THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Eugene W. Holowachuk

TITLE OF THESIS A study of genetic fine structure and complementation at the methionine repressor gene (*metJ*) of *Escherichia coli*

DEGREE FOR WHICH THESIS WAS PRESENTED Master of Science

YEAR THIS DEGREE GRANTED 1976

THE UNIVERSITY OF ALBERTA

A STUDY OF GENETIC FINE STRUCTURE AND COMPLEMENTATION AT THE METHIONINE REPRESSOR GENE (*metJ*) OF *Escherichia coli*

by

EUGENE W. HOLOWACHUK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

SPRING, 1976

THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A study of genetic fine structure and complementation at the methionine repressor gene (*metJ*) of *Escherichia coli* submitted by Eugene W. Holowachuk in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The biosynthesis of methionine from aspartate is catalyzed by eight enzymes coded by *met* structural genes which are non-contiguously arranged on the *Escherichia coli* chromosome. The expression of these genes is subject to non-coordinate repression by methionine. This repression is brought about by the *met* repressor which is known to be specified by the *metJ* gene.

Several *metJ* mutants were selected as mutants resistant to the methionine analog, ethionine. These mutants were characterized by close linkage to the *metB* locus and elevated levels of the *met* biosynthetic enzymes. The *metJ* locus was found to map at min 78 in the clockwise order *glpK metB metJ metL,M metF argH* on the genetic map of *E. coli* K12. A fine structure genetic map of the *metJ* gene, showing the order of 20 sites of mutation (including 16 non-*amber* and 4 *amber metJ* alleles), was constructed by four-point transduction crosses.

The dominance of *metJ* mutants was tested by the construction of $metJ^+/metJ^-$ merodiploids. Three mutants, viz., $metJ65_{am}$, *metJ1271*, and *metJ1275*, exhibited dominance as judged by ethionine resistance, elevated levels of *metA, metB, metC,* and *metK* enzymes, and over-production of methionine. The *trans*-dominance of these *metJ* mutants is probably due to subunit interactions which is consistent with the interpretation that the *met* repressor is an oligomeric protein. The map positions of the dominant mutations within the *metJ* gene ($metJ65_{am}$ and *metJ1275* map at the left end of *metJ*, whereas *metJ1271*

maps toward the right) suggest that both the amino- and carboxy-termini of the repressor may be crucial to its regulatory function.

Complementation at the *metJ* locus was studied by the construction of *metJ/metJ* merodiploids in various pairwise combinations between 16 *metJ* mutants carried on the episome and 20 mutants carried on the chromosome. Although genotypically *metJ/metJ*, several of the merodiploids became ethionine sensitive indicating the occurrence of positive complementation. The evidence indicates that complementation between *metJ* mutants is intra-cistronic which typically involves protomer-protomer interactions.

The regulatory behavior displayed by complementing *metJ/metJ* merodiploids was of two distinct kinds: (i) merodiploids in which the repression of the *metA, metB, metC,* and *metK* genes was completely restored; and (ii) merodiploids which exhibited *restrictive* complementation resulting in the selective repression of the *metA* gene (without affecting the remaining genes). Both kinds of merodiploids produced normal levels of intra-cellular and extra-cellular methionine. The merodiploids exhibiting restrictive complementation regained ethionine resistance when supplied with exogenous cystathionine or homocysteine. These results indicate that the aggregation of mutationally altered *metJ* protomers with other mutant protomers, or even *amber* fragments, can lead to the formation of hybrid repressors which are fully or partially functional in repression. The selective repression of the *metA* gene, observed in some cases, suggests that either the operator site of this gene is

different from other *met* structural genes, or that this gene is subject to an additional regulatory mechanism which does not operate on other *met* genes.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Doctor A. Ahmed, for his guidance and helpful criticism during the course of this investigation.

Hanna Morowicz and Chris Somerville are gratefully acknowledged for providing numerous helpful suggestions and stimulating discussions.

I would like to thank my parents for their constant support and encouragement during the course of this study.

Appreciation is extended to Mrs. M. Brennan for skillful typing of this thesis.

Finally, I gratefully thank Judy Gray for her constant encouragement, moral support, and understanding throughout the course of this study.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

INTRODUCTION

Extensive studies of the system for lactose utilization in *Escherichia coli* have led to the formulation of a unified hypothesis for the control of gene expression (Jacob and Monod, 1961). The basic transcriptive unit of the Jacob-Monod model, the operon, was shown to be a cluster of contiguous structural genes which specify related functions. The expression of the lactose (*lac*) operon was found to be regulated in a negative manner by a cytoplasmic repressor. The repressor acts on a site (the operator) on the DNA, in response to cellular concentrations of the inducer (lactose), to control transcription of the operon by RNA polymerase. This model has been supported subsequently by extensive genetic and biochemical studies on the *lac* operon (Beckwith, 1964; Bourgeois, Cohn, and Orgel, 1965; Gilbert and Müller-Hill, 1966; Miller *et al.*, 1968). More recent investigations have shown that the *lac* operon is also subject to a positive system of regulation. This system involves the catabolite gene activating protein (CAP) which activates the transcription of the *lac* operon, in addition to other catabolic operons sensitive to catabolite repression, in conjunction with 3':5'-cyclic AMP (cAMP) (Zubay, Schwartz, and Beckwith, 1970; Emmer *et al.*, 1970; Schwartz and Beckwith, 1970).

Several attempts have been made to apply the Jacob-Monod model of gene regulation to various biosynthetic systems. Early experiments by Cohen and Jacob (1959) demonstrated that resistance

to various structural analogs of a metabolic end product is a common phenotype of bacterial strains harboring regulatory mutations in that particular biosynthetic pathway. Thus, a mechanism was provided for the isolation and characterization of mutants altered in the regulation of metabolic pathways, and was subsequently utilized for the isolation of such mutants in systems like the tryptophan and histidine biosynthetic pathways.

The mechanism of repression has been studied in detail for the tryptophan (*trp*) operon of *E. coli* and the histidine (*his*) operon of *Salmonella typhimurium*. Studies of Yanofsky and associates have shown that a repressor protein, specified by the *trpR* gene (Morse and Yanofsky, 1969; Zubay *et al.*, 1972), regulates the expression of the *trp* operon. Repression is brought about by repressor-operator interactions which prevent the binding of the RNA polymerase to initiate transcription (Rose *et al.*, 1973; Shimizu *et al.*, 1973; Squires *et al.*, 1975). An independent regulatory mechanism termed "attenuation" also seems to operate on the expression of the *trp* operon. In this process, a factor, together with tryptophan, regulates the termination of *trp* messenger RNA synthesis in the attenuator region of the operon which is located just before the structural genes (Bertrand *et al.*, 1975). Deletions of the attenuator result in enhancement of operon expression in *trpR* background.

Investigations by Ames and associates have established that histidyl-$tRNA^{His}$ probably acts as the corepressor in the control of the expression of the *his* operon. This conclusion is

based on the findings that mutants defective in the synthesis, modification, or aminoacylation of tRNA^{His} are derepressed (Brenner and Ames, 1971, 1972; Singer *et al.*, 1972; De Lorenzo *et al.*, 1972). In the absence of direct evidence for a repressor-operator regulatory mechanism for the *his* operon, the major control process appears to be a positive activator-attenuator mechanism (Artz and Broach, 1975). Evidence indicates that a positive factor interacts with the *his* operator region which permits transcription beyond the attenuator in a manner similar to the *trp* operon. Thus, inactivation of the positive factor by histidyl-tRNA^{His} could control the expression of the *his* operon. In addition, guanosine tetraphosphate (ppGpp) seems to act as an additional positive effector for *his* operon transcription as a general signal for amino-acid deficiency (Stephens *et al.*, 1975).

The examples described above represent only a few of the many investigations into mechanisms of gene regulation in prokaryotes. Comprehensive reviews of control mechanisms in bacteria can be found in articles by Umbarger (1971), Reznikoff (1972), Beckwith and Rossow (1974), and Gots and Benson (1974).

Genes and enzymes of methionine biosynthesis

In view of the observed differences in the mechanisms of regulation of the *trp* and *his* operons, it is obvious that studies with other amino acid biosynthetic systems are desirable to understand the mechanisms of repression. A study of methionine biosynthesis is of interest from the genetic point of view because the biosynthetic

enzymes are coded by structural genes which are scattered, i.e., arranged non-contiguously, on the chromosome. From the point of cell physiology, methionine plays a central role in the initiation and assembly of proteins, in various methylation reactions via the synthesis of *S*-adenosylmethionine, in the synthesis of polyamines by donating the propylamine moiety, in chemotaxis via the synthesis of *S*-adenosylmethionine, and it also contributes the methyl group of choline for membrane phospholipid. Thus, the regulatory mechanisms for methionine biosynthesis are likely to be complex. Detailed reviews of the genetic and biochemical aspects of methionine biosynthesis have been published by Smith (1971) and Flavin (1975). The essential features of this system are outlined below.

Methionine is a member of the family of amino acids derived from aspartate. The conversion of aspartate to methionine (Figure 1) is catalyzed by the enzymes whose structural genes are scattered on the *E. coli* genetic map (Figure 2). The *metA* and *metH* genes are located at min 79.5; *metB*, *metL,M*, and *metF* form a cluster at min 78; *metC* maps at min 58; and *metE* maps at min 75.5.

Initially, aspartate is phosphorylated to aspartyl β-phosphate by β-aspartokinase II (*metL*) using ATP as the phosphate donor. Aspartyl β-phosphate is dephosphorylated and reduced by aspartate β-semialdehyde dehydrogenase to form aspartate β-semialdehyde. The enzyme homoserine dehydrogenase II (*metM*) transforms aspartate β-semialdehyde to homoserine. Aspartokinase II and homoserine dehydrogenase II form a complex with a molecular weight of 300,000 (Cohen, 1969), and it has been suggested that the

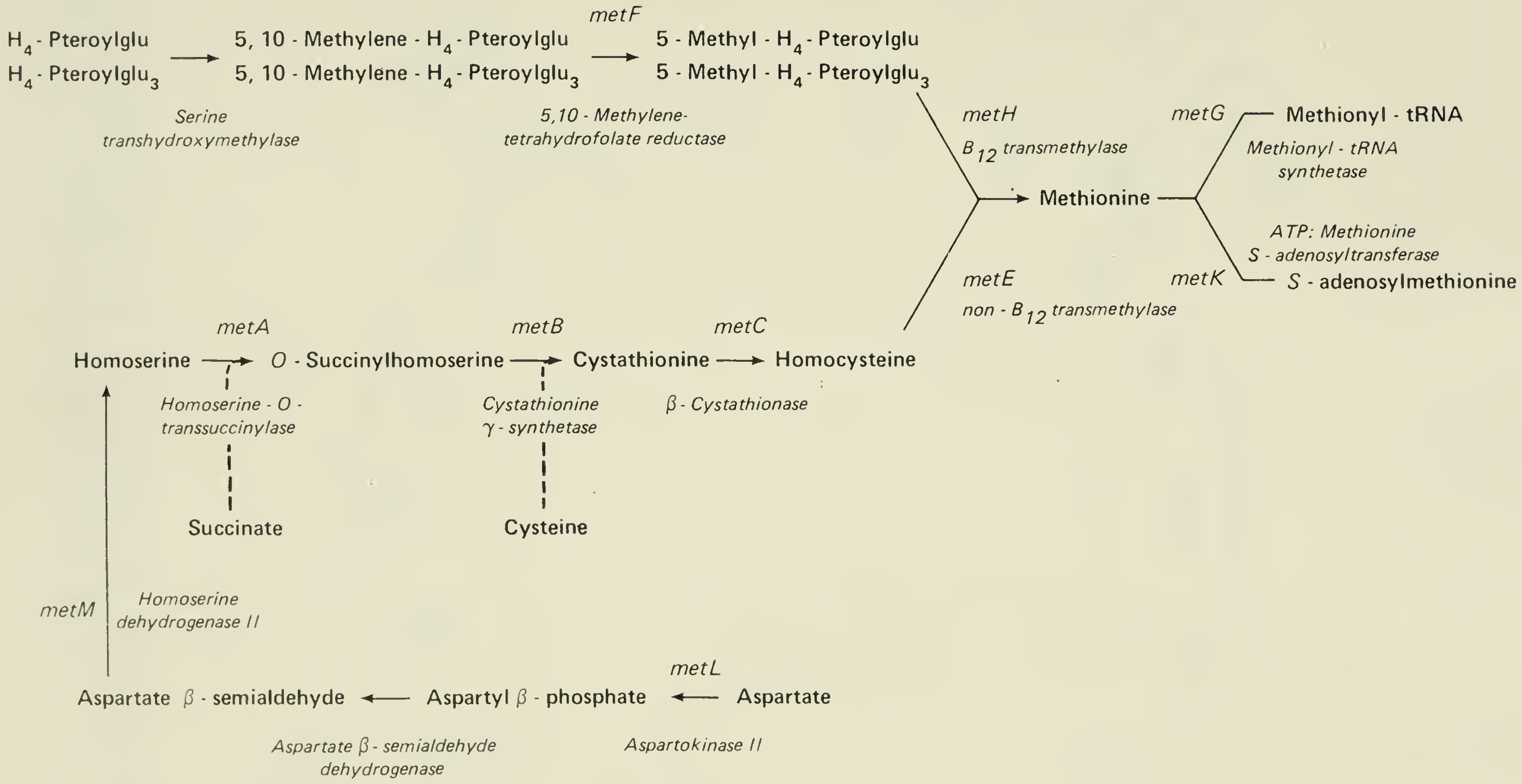

FIGURE 1 - The pathway for biosynthesis and utilization of methionine in *E. coli*.

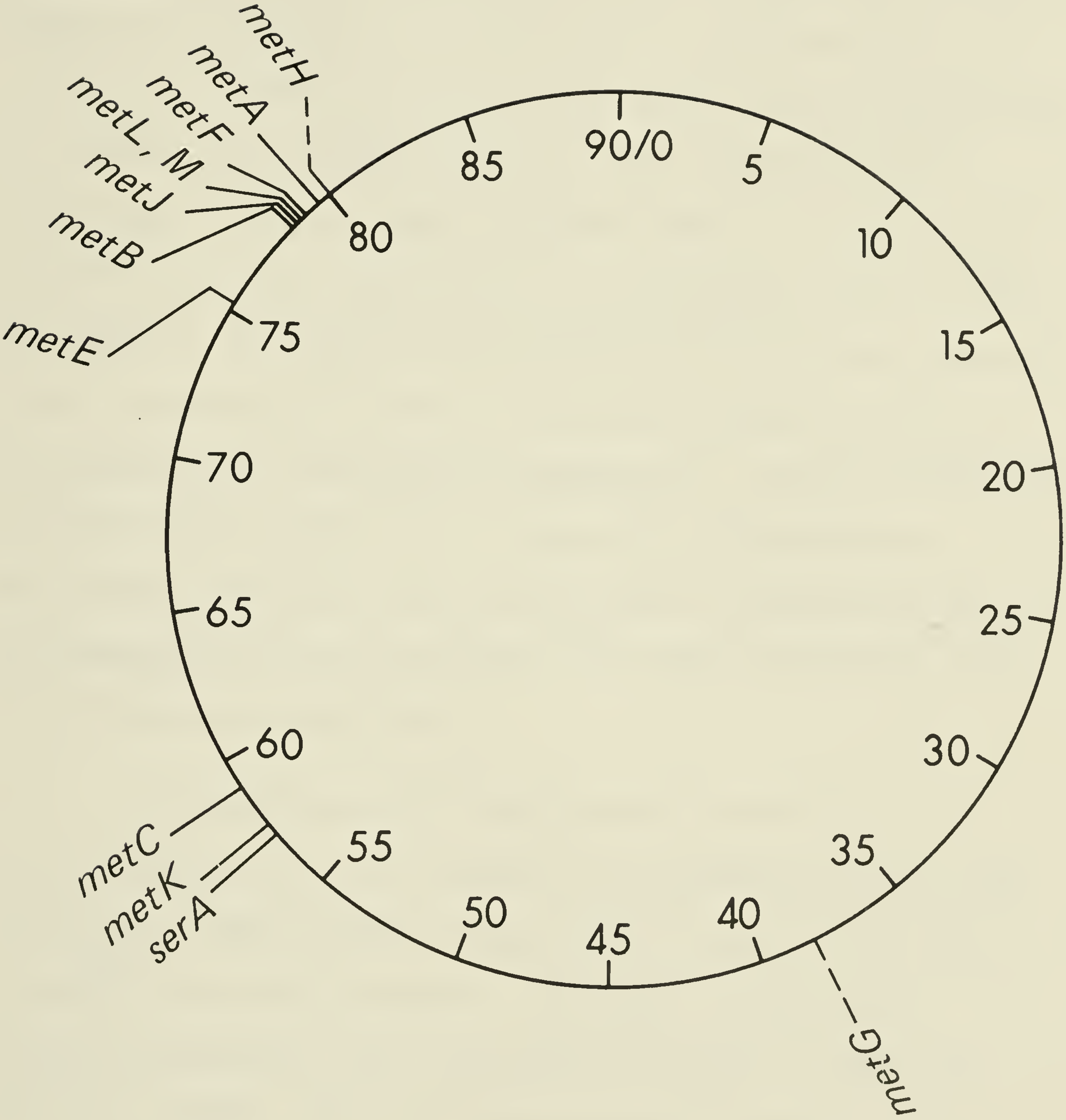

FIGURE 2 - Genetic map of *E. coli* showing the location of the structural and regulatory genes of methionine biosynthesis. Broken lines indicate approximate locations of the genes.

two activities reside in a single polypeptide (Theze *et al.*, 1974). The first specific precursor of methionine, viz., *O*-succinylhomoserine, is synthesized by the enzyme homoserine-*O*-transsuccinylase (*metA*) which succinylates homoserine in the presence of succinate, ATP, and coenzyme A. In the next step, the succinyl group of *O*-succinylhomoserine is replaced by cysteine in a trans-sulfuration reaction catalyzed by cystathionine-γ-synthetase (*metB*) to yield cystathionine. This enzyme is an oligomer comprised of four identical subunits with a total molecular weight of 160,000 (Kaplan and Flavin, 1966). Cystathionine is then hydrolyzed by β-cystathionase (*metC*) to yield homocysteine. The enzyme N^5,N^{10}-methylenetetrahydrofolate reductase (*metF*) carries out the synthesis of the methyl group required for the methylation of homocysteine to methionine. The actual methylation of homocysteine to methionine is catalyzed by N^5-methyltetrahydropteroyl triglutamate-homocysteine methyl transferase (*metE*), or by N^5-methyltetrahydrofolate-homocysteine methyl transferase (*metH*) in the presence of vitamin B12.

The methionine thus formed can participate directly in protein synthesis or in the synthesis of *S*-adenosylmethionine (SAM). The methionyl-tRNA synthetase (*metG*) can aminoacylate both species of $tRNA^{Met}$ for protein synthesis. The enzyme ATP:Methionine *S*-adenosyltransferase (*metK*) catalyzes the synthesis of SAM from methionine and ATP. SAM participates directly in various transmethylation reactions (Cantoni, 1965) and in polyamine biosynthesis (Tabor *et al.*, 1961) following decarboxylation.

Regulation of methionine biosynthesis

The synthesis of methionine biosynthetic enzymes and ATP: Methionine *S*-adenosyltransferase is repressed in a non-coordinate manner by the addition of methionine to cultures of *S. typhimurium* (Lawrence *et al.*, 1968) and *E. coli* (Holloway *et al.*, 1970). Moreover, the first enzyme of methionine biosynthesis, viz., homoserine-*O*-transsuccinylase, is also subject to cooperative feedback inhibition by methionine and SAM (Lee *et al.*, 1966). These results indicate that both repression and feedback inhibition are operational in the control of methionine biosynthesis.

The regulation of methionine biosynthesis was first investigated in *S. typhimurium* by Lawrence *et al.*, (1968). They described three classes of regulatory mutants (*metI*, *metJ*, and *metK*) which were resistant to the methionine analogs ethionine, α-methylmethionine, and norleucine. Similar mutants were reported in *E. coli* by Ahmed and Duncan (1968), and described in detail by Greene *et al.*, (1970), Holloway *et al.*, (1970), and Ahmed (1973).

The *metI* mutants were resistant to α-methylmethionine and ethionine, mapped within the *metA* gene, and contained homoserine-*O*-transsuccinylase which was insensitive to feedback inhibition (Chater and Rowbury, 1970). Deletion mapping and complementation studies of these mutants indicated that the *metA* gene probably specifies a single polypeptide which contains both one catalytic and one regulatory site (Smith and Childs, 1966; Chater and Rowbury, 1970). The *metI* mutants overproduced methionine, but remained

repressible by methionine and display normal levels of the biosynthetic enzymes (Smith, 1971).

The *metJ* mutants were resistant to ethionine and were closely linked to the *metB* locus. These mutants exhibited constitutive (i.e., derepressed) levels of the methionine biosynthetic enzymes and somewhat elevated levels of ATP:Methionine *S*-adenosyltransferase. Studies with $metJ^+/metJ^-$ merodiploids established that the *metJ* gene exerts its effects in a negative manner through the formation of a cytoplasmic repressor product (Chater, 1970; Su and Greene, 1971; Ahmed, 1973), rather than by the alteration or modification of methionyl-tRNA^{Met} (Ahmed, 1973). The isolation of nonsense *metJ* mutants of *S. typhimurium* by Minson and Smith (1972) and *E. coli* by Morowicz (1975), and the demonstration of dominance of some *metJ* mutants in $metJ^+/metJ^-$ merodiploids (Chater, 1970; Morowicz, 1975) indicated that the repressor specified by the *metJ* gene is an oligomeric protein.

The third class of regulatory mutants, *metK*, was resistant to ethionine and was found to be linked to the *serA* locus. These mutants were found to contain reduced levels of ATP:Methionine *S*-adenosyltransferase (Greene *et al.*, 1970; Ahmed, 1973; Hobson and Smith, 1973) and several nonsense *metK* mutants have been identified (Minson and Smith, 1972; Morowicz, 1975). Hobson and Smith (1973) reported the existence of two kinds of *metK* mutants in *S. typhimurium*. The first class consisted of mutants which were repressible by methionine, did not overproduce methionine, and contained ATP: Methionine *S*-adenosyltransferase displaying an altered affinity for

methionine. The second class of *metK* mutants exhibited elevated levels of methionine biosynthetic enzymes, overproduced methionine, and contained no detectable ATP:Methionine *S*-adenosyltransferase activity. Hobson (1974) subsequently reported a *metK* mutant which contained normal ATP:Methionine *S*-adenosyltranferase activity and showed complementation with other *metK* mutants.

It can be concluded from the findings described above that the *metJ* gene codes for the *met* repressor which is an oligomeric protein and controls methionine biosynthesis in a negative manner. Possibly, the repressor interacts with operator sequences located adjacent to the *met* structural genes. However, no evidence pointing to the existence of such operator sequences is available. The exact nature of the corepressor is not clear, but the evidence indicates that SAM, or a derivative of SAM, could act as the corepressor. Finally, the possibility remains that the *metK* enzyme itself is also involved in the repression mechanism.

The present work represents a detailed genetic study of the *metJ* gene which specifies the *met* repressor. Several *metJ* mutants were isolated and characterized. A fine structure genetic map showing the order of mutational sites within the *metJ* gene was constructed, and the complementation behavior and dominance of these mutants was studied. Finally, the implications of these findings on the nature and the function of the *met* repressor have been discussed.

MATERIALS AND METHODS

Bacterial and bacteriophage strains

The genotypes and sources of the strains used in this study, all of which were derived from *E. coli* K12, are described in Table 1. The bacteriophages $T4^+$, an *amber* mutant of T4 (T4*am*), and the generalized transducing phage P1*vir* were obtained from Dr. A. Ahmed's collection. Phage $\phi 80psu3^+su3^-h^-$ was supplied by Dr. N. Franklin and Dr. S. Adhya. *Leuconostoc mesenteroides* P60 was obtained from Dr. E. A. Cossins.

Media

The medium of Davis and Mingioli (as described by Roth, 1970) containing 0.2% glucose, lactose, or 2% glycerol was used as the minimal medium. This medium was supplemented, when necessary, with 20 μg/ml of the required L-amino acid, 10 μg/ml of thiamine HCl, and 0.1 μg/ml of vitamin B12. Ethionine (L-, or DL- form) was used at 3 mg/ml for scoring ethionine resistance. L Broth or L agar (Roth, 1970) was used as the complete medium.

Isolation and classification of L-ethionine resistant mutants

Spontaneous L-ethionine resistant mutants of the AA1200 series were isolated from the parental strain CSH4 (F^- $lacZ_{am}$ trp_{am} *strA thi* su^o_{am}). Ninety parallel cultures inoculated with single colonies were grown to saturation in L broth at 30°. Aliquots of 10 - 20 μl of each culture were applied on 1/8 sectors on minimal plates containing tryptophan and 3 mg/ml L-ethionine. After 48 hrs

TABLE 1 - Bacterial strains

Strain	Genotype	Source
CSH4	F^- $lacZ_{am}$ trp_{am} *strA thi* su^{o}_{am}	Cold Spring Harbor Strain Kit
AA1201-AA1290	F^- $lacZ_{am}$ trp_{am} *strA thi* su^{o}_{am}	L-ethionine resistant mutants of CSH4
B36	Hfr H *proB metB36 thi* su^{o}_{am}	A. Ahmed collection, University of Alberta
AT2475	Hfr H *serA thi* su^{o}_{am}	" " " "
J36	Hfr H *proB metJ36 thi* su^{o}_{am}	" " " "
J101	Hfr H *proB metJ101 thi* su^{o}_{am}	" " " "
J120	Hfr H *proB metJ120 thi* su^{o}_{am}	" " " "
HM65	Hfr C $lacZ_{am}$ trp_{am} $metJ65_{am}$ *thi* su^{o}_{am}	H. Morowicz, University of Alberta
HM89	Hfr C $lacZ_{am}$ trp_{am} $metJ93_{am}$ *thi* su^{o}_{am}	" " "
HM93	Hfr C $lacZ_{am}$ trp_{am} $metJ93_{am}$ *thi* su^{o}_{am}	" " "
HM1148	F^- $lacZ_{am}$ trp_{am} *strA metJ148 thi* su^{o}_{am}	" " "
R1	F^- *strA mtl malA ilv glpK metB argH thi* su^{+}_{am}	" " "
GH311	F^- *strA mtl malA ilv glpK argH thi* su^{+}_{am}	Transduction of R1 to $metB^+$
GH350	F^- *strA mtl malA ilv glpK metJ36 argH thi* su^{+}_{am}	Transduction of R1 to $metB^+$ $metJ^-$
GH330	" " " " " " *metJ101* " " "	" " " "
GH306	" " " " " " *metJ120* " " "	" " " "
GH320	" " " " " " *metJ148* " " "	" " " "
GH361	" " " " " " *metJ1245* " " "	" " " "
GH362	" " " " " " *metJ1249* " " "	" " " "
GH364	" " " " " " *metJ1252* " " "	" " " "
GH366	" " " " " " *metJ1256* " " "	" " " "

Table 1 - continued

Strain	Genotype	Source
GH369	F^- *strA mtl malA ilv glpK metJ1257 argH thi* su^+_{am}	Transduction of R1 to metB$^+$ metJ$^-$
GH371	" " " " " " *metJ1258* " " "	" " " "
GH375	" " " " " " *metJ1264* " " "	" " " "
GH377	" " " " " " *metJ1271* " " "	" " " "
GH378	" " " " " " *metJ1275* " " "	" " " "
GH380	" " " " " " *metJ1277* " " "	" " " "
GH383	" " " " " " *metJ1279* " " "	" " " "
GH384	" " " " " " *metJ1284* " " "	" " " "
161	F^- *his strA mtl malA ilv glpK metB argH thi* su^+_{am}	E.C.C. Lin, Harvard University
Hfr H 3000	Hfr H *thi* su^o_{am}	B. Bachmann, Yale University
R2	F^- *strA mtl malA ilv glpK metB argH thi* su^o_{am}	Mating between Hfr H 3000 and 161
GH390	F^- *strA mtl malA ilv glpK* $metJ1209_{am}$ *argH thi* su^o_{am}	Transduction of R2 to metB$^+$ metJ$^-$
GH392	" " " " " " $metJ89_{am}$ " " "	" " " "
GH394	" " " " " " $metJ93_{am}$ " " "	" " " "
GH396	" " " " " " $metJ65_{am}$ " " "	" " " "
GH398	F^- *strA mtl malA ilv glpK argH thi* su^o_{am}	Transduction of R2 to metB$^+$

TABLE 1 - continued

Strain	Genotype	Source
AB1206	F14/Δ(*ilv metBJF arg*) *proA lacY galK his strA tfr thi* su^{+}_{am}	B. Bachmann, Yale University
GH418	F14 *metB*/Δ(*ilv metBJF arg*) *proA lacY galK his strA tfr thi* su^{+}_{am}	*metB* mutant derived from AB1206
GH420	F14/Δ(*ilv metBJF arg*) *proA lacY galK his strA tfr thi* su^{+}_{am}	Transduction of GH418 to metB^{+}
GH430	F14 *metJ120*/Δ(*ilv metBJF arg*) *proA lacY galK his strA tfr thi* su^{+}_{am}	metB^{+} metJ^{-} transductant of GH418
GH431	F14 *metJ101*/ " " " " " " " " " " "	" " " "
GH436	F14 *metJ36*/ " " " " " " " " " " "	" " " "
GH438	F14 *metJ148*/ " " " " " " " " " " "	" " " "
GH445	F14 *metJ1245*/ " " " " " " " " " " "	" " " "
GH449	F14 *metJ1249*/ " " " " " " " " " " "	" " " "
GH452	F14 *metJ1252*/ " " " " " " " " " " "	" " " "
GH456	F14 *metJ1256*/ " " " " " " " " " " "	" " " "
GH457	F14 *metJ1257*/ " " " " " " " " " " "	" " " "
GH458	F14 *metJ1258*/ " " " " " " " " " " "	" " " "
GH464	F14 *metJ1264*/ " " " " " " " " " " "	" " " "
GH471	F14 *metJ1271*/ " " " " " " " " " " "	" " " "
GH475	F14 *metJ1275*/ " " " " " " " " " " "	" " " "
GH477	F14 *metJ1277*/ " " " " " " " " " " "	" " " "
GH479	F14 *metJ1279*/ " " " " " " " " " " "	" " " "
GH484	F14 *metJ1284*/ " " " " " " " " " " "	" " " "

incubation at 37°, several ethionine resistant colonies appeared in each sector. A single colony from each culture was taken through two single colony purifications on minimal plates with ethionine. Stocks of these mutants were maintained on LB plates and on appropriate minimal plates lacking ethionine. No difficulties, such as loss of the ethionine resistant phenotype, were encountered with these mutants. These mutants were found to be equally resistant to inhibition by L- or DL-ethionine at 37°. After the initial isolation, the ethionine resistant phenotype of the mutants was routinely scored on DL-ethionine instead of the L- form.

The 90 ethionine resistant mutants were classified as methionine regulatory mutants (*metJ* or *metK*) or nonregulatory mutants. P1*vir* lysates of all of the mutants were prepared (according to the method described by Roth, 1970) and used to transduce the strains, B36 (Hfr H *proB metB36 thi* su^{o}_{am}) and AT2475 (Hfr H *serA thi* su^{o}_{am}), to $metB^{+}$ and $serA^{+}$, respectively. From each cross, 50 $metB^{+}$ and 50 $serA^{+}$ transductants were picked and tested for cotransduction of ethionine resistance on minimal plates (supplemented as required) containing 3 mg/ml DL-ethionine. The *metJ* mutants were identified by their tight linkage to *metB*, and the *metK* mutants were recognized by their cotransducibility with the *serA* gene. The nonregulatory mutants were cotransducible with neither *metB* nor *serA*. This preliminary characterization of the ethionine resistant mutants was subsequently confirmed by enzyme assays.

Genetic mapping

Mutants were mapped by generalized transduction with phage P1*vir* according to the procedures described by Lennox and Yanofsky (1959).

Identification of ethionine resistant mutants carrying amber mutations

Bacteriophage ϕ80p*su3*$^+$ carries the structural gene for suppressor $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ which efficiently suppresses the *amber lac* and *trp* mutations present in CSH4. The AA1200 series of ethionine resistant mutants were lysogenized at 37° with ϕ80p*su3*$^+$ by transferring patches of mutant cells with toothpicks onto glucose minimal plates lacking tryptophan and seeded with 10^9 phage. The trp$^+$ colonies were purified and tested for suppression of *lac* on lactose minimal plates. All of the trp$^+$ colonies were also found to be lac$^+$ due to lysogenization with ϕ80p*su3*$^+$. These lysogens were then tested for sensitivity to DL-ethionine. Those found to be sensitive to DL-ethionine after 48 hrs incubation at 37° were classified as *amber* ethionine resistant mutants. The lysogens which remained resistant were grouped as non-*amber* and probably include other kinds of mutants such as missense, *ochre*, frame shift, etc. Some of these *amber* ethionine resistant mutants were assayed for cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase activities in the suppressed and nonsuppressed conditions to determine suppression.

Isolation of spontaneous su^{+}_{am} *derivatives of amber ethionine resistant mutants*

The *su*$^{+}$ derivatives of the *amber* ethionine resistant mutants in the AA1200 series were selected by plating 0.2 ml of saturated L broth cultures onto lactose minimal plates followed by 48 hrs incubation at 37°. Spontaneous lac^{+} trp^{+} colonies were purified and tested for the presence of *amber su*$^{+}$ by their ability to support the growth of T4*am*. The *su*$^{+}$ derivatives were then tested for suppression of the *amber* ethionine resistant mutation, present in the strains, by the restoration of sensitivity to DL-ethionine. Enzyme activities of cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase were determined for selected strains.

Construction of strain R2

The strain R2 is a *su*^{o} derivative obtained from a conjugal cross between Hfr H 3000 (*thi* su^{o}_{am}) and 161 (F^{-} *his strA mtl malA ilv glpK metB argH thi* su^{+}_{am}). Fifty his^{+} str^{r} recombinants, selected from this cross, were purified and tested for su^{o}_{am} on LB plates seeded with 10^9 T4^{+} or 10^9 T4*am* phage. Twelve his^{+} str^{r} recombinants were found to be su^{o}_{am} and their plating efficiencies of bacteriophage T4 and T4*am* were determined. One *su*^{o} recombinant was selected and designated strain R2.

Construction of metJ derivatives of strains R1 and R2

P1*vir* lysates were prepared from 20 *metJ* mutants (glpK^{+} metB^{+} metJ^{-} argH^{+}). Sixteen of these *metJ* mutants, which were not

suppressible by su^{+}_{am}, were transduced into the strain R1 (*ilv glpK metB argH* su^{+}_{am}). The remaining 4 *metJ* mutants, which were *amber*, were introduced into the strain R2 (*ilv glpK metB argH* su^{o}_{am}). In both cases, this was achieved by selection of $metB^{+}$ transductants on appropriate plates. For each *metJ* allele used, 100 $metB^{+}$ transductants were tested for the genetic markers ilv, glpK, metJ, and argH. Transductants having the genotype *ilv glpK metJ argH* were designated the GH300 series. The identity of each *metJ* allele was confirmed by transducing each derivative to $argH^{+}$ or $glpK^{+}$ with the original P1*vir* lysate of the *metJ* allele. In each case, 100 transductants were tested for ethionine sensitivity (i.e., $metJ^{+}$) and none was found. The activities of cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase, of these strains, were determined. The complete genotypes of these *metJ* derivatives are summarized in Table 1. These strains were used for fine structure mapping of the *metJ* gene, dominance studies, and for complementation analysis in *metJ/metJ* merodiploids described in the succeeding sections.

Orientation of metJ with respect to glpK, metB, and argH

The position of the *metJ* gene with respect to the *glpK*, *metB*, and *argH* loci was determined with four-point transduction crosses. The strain R1 (*glpK metB* $metJ^{+}$ *argH*) was transduced with P1*vir* lysates prepared on several *metJ* strains (of the genotype $glpK^{+}$ $metB^{+}$ $metJ^{-}$ $argH^{+}$). $MetB^{+}$ transductants were selected, purified, and scored for $glpK^{+}$, $metJ^{-}$, and $argH^{+}$ markers. The number of transductants tested varied from 300 to 1000 for the different crosses.

Fine structure mapping of the metJ gene

The sequence of 20 *metJ* alleles, including 4 *amber metJ* alleles, was determined by four-point transduction crosses. P1*vir* lysates, grown on glpK$^+$ metJ$^-$ argH$^+$ strains, were used to transduce the *metJ* derivatives of strains R1 and R2. ArgH$^+$ and glpK$^+$ transductants were selected on glucose and glycerol minimal plates containing appropriate supplements. The transductants, varying in number from 200 to 500, were purified and scored for argH$^-$, glpK$^-$, and metJ$^-$ markers.

Episomal transfer

F' transfers were performed by spotting together 0.05 ml of each of the donor and recipient ($\sim 10^9$ cells/ml) on a minimal plate selecting for recipient cells which had acquired the episome. Colonies picked from the mixed donor-recipient spot were purified by single colony isolations on the same plates before other genetic markers were tested.

Isolation of a metB mutant (GH418) from strain AB1206

Methionine auxotrophs of strain AB1206 (F14/Δ(*ilv metBJF arg*) su^+_{am}) were isolated by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). Of the 434 mutagenized colonies tested, 37 were found to require methionine. These methionine auxotrophs were classified by their growth response to various methionine intermediates. A *metB* mutant which retained the ability to donate F14 was designated GH418 and selected for further work. This strain harbors the *metB*

mutation on the F14 episome and was employed for the construction of *metJ* derivatives used as donors for complementation studies.

Construction of F14 metJ derivatives

P1*vir* lysates of 16 non-*amber metJ* alleles were used to transduce the strain GH418, and metB$^+$ colonies were selected. One hundred metB$^+$ colonies were picked from each cross and tested for cotransduction of the *metJ* allele on appropriate glucose minimal plates containing DL-ethionine. The metJ$^-$ phenotype was scored after 48 hrs incubation at 37°. One *metJ* transductant from each cross was purified, retested for various genetic markers, and incorporated into the GH400 series. The identity of each F14 *metJ* derivative was confirmed by P1 transduction of the corresponding R1 *metJ* derivative to argH$^+$ or glpK$^+$ with phage grown on the F14 *metJ* derivatives. In each case, 100 transductants were then tested for ethionine sensitivity (i.e., metJ$^+$) and none was found. This was taken as evidence to show that the *metJ* alleles in the two strains were the same. The specific activities of cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase were also determined for each F14 *metJ* strain. These F14 *metJ* derivatives were used as donors for complementation studies in *metJ/metJ* merodiploids.

Determination of dominance of metJ alleles in metJ$^-$/metJ$^+$ merodiploids

The dominance of the *metJ* mutants included in this study was examined by the construction of *metJ*$^-$/*metJ*$^+$ merodiploids. F14 episomes bearing the non-*amber metJ* alleles were transferred into strain GH311

(F$^-$ *ilv glpK metJ*$^+$ *argH su*$^+_{\mathrm{am}}$). In the reciprocal combination, F14 bearing *metJ*$^+$ was transferred into *metJ* derivatives of R1 and R2. All of the 16 non-*amber metJ* derivatives of strain R1 had the genotype: F$^-$ *ilv glpK metJ argH su*$^+_{\mathrm{am}}$. The 4 *amber metJ* alleles were introduced into R2, and had the same genotype except that these were *su*$^o_{\mathrm{am}}$. The episomal markers *ilv*$^+$, *glpK*$^+$, and *argH*$^+$ were used for the selection of *metJ*$^-$/*metJ*$^+$ merodiploids by spot matings as described earlier. Episome transfer was followed initially by the selection of ilv$^+$ arg$^+$ on glucose minimal plates, and the F-ductants were tested subsequently for glpK$^+$ on glycerol minimal plates. In this manner, merodiploids with the following genotypes were constructed: F14 *ilv*$^+$ *glpK*$^+$ *metJ*$^-$ *argH*$^+$/*ilv*$^-$ *glpK*$^-$ *metJ*$^+$ *argH*$^-$ and F14 *ilv*$^+$ *glpK*$^+$ *metJ*$^+$ *argH*$^+$/ *ilv*$^-$ *glpK*$^-$ *metJ*$^-$ *argH*$^-$. Two ilv$^+$ glpK$^+$ argH$^+$ merodiploids from each mating were tested for dominance of the *metJ*$^-$ allele by determining the growth response of the *metJ*$^-$/*metJ*$^+$ merodiploids on ethionine. In cases where the merodiploids were ethionine resistant, suggesting dominance of the *metJ*$^-$ allele, the merodiploids were reconstructed up to five times in order to confirm the observed phenotype. The activities of cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase were determined for the merodiploids which exhibited dominance reproducibly. Some of these merodiploids were also assayed for homoserine-*O*-transsuccinylase.

Complementation studies in metJ/metJ merodiploids

Intracistronic complementation in *metJ/metJ* merodiploids was examined by transferring F14 harboring the 16 non-*amber metJ* alleles into *metJ* derivatives of the strains R1 (having the same *metJ* alleles in a su^+ background) and R2 (having 4 *amber metJ* alleles in su^o background). The recipient strains carried the markers *ilv glpK metJ argH*, and the F14 episome carried ilv^+, $glpK^+$, $metJ^-$, and $argH^+$. Episome transfers were performed by spot matings, and ilv^+ $glpK^+$ $argH^+$ F-ductants were selected, as described in the previous section. The *metJ/metJ* merodiploids, thus constructed, had the genotype F14 ilv^+ $glpK^+$ $metJ^-$ $argH^+/ilv^-$ $glpK^-$ $metJ^-$ $argH^-$, and all possible pairwise combinations of various *metJ* alleles were prepared. Two *metJ/metJ* merodiploids from each mating were tested for complementation by determining their growth response on ethionine. Restoration of ethionine sensitivity in the merodiploid was considered as positive complementation, whereas ethionine resistance indicated lack of complementation. The *metJ/metJ* merodiploids found to be ethionine sensitive were reconstructed several times to confirm the observed phenotype. The complementing merodiploids were assayed for the enzymes cystathionine-γ-synthetase, β-cystathionase, and ATP: Methionine *S*-adenosyltransferase. Some of these merodiploids were also assayed for homoserine-*O*-transsuccinylase.

Growth of bacteria for enzyme assays

Cells grown to stationary phase in 5 ml minimal medium (with desired supplementation) were inoculated directly into 20 ml

of the same medium and grown overnight at 30°. The bacteria were centrifuged, resuspended in 250 ml fresh minimal medium, and grown with vigorous aeration for approximately 6 hrs at 30°. On reaching late log phase, the culture flasks were rapidly chilled in an ice bath, and the cells were harvested by centrifugation. The cell pellet was washed with 10 ml of 50 mM potassium phosphate buffer at pH 7.3 and stored at -40° until assayed.

On occasions when large numbers of strains were to be assayed, cells from 5 ml overnight cultures in minimal medium were centrifuged and resuspended in 50 ml fresh minimal medium. The cells were harvested in late log phase, washed and used immediately or stored frozen for future use.

Preparation of cell free extracts

The cell pellets were suspended in 1 - 2 ml of 50 mM potassium phosphate buffer at pH 7.3, and disrupted by two 30 sec bursts of a Branson S125 sonifier at medium intensity in ice with 1 min cooling period in between. The disrupted cell suspensions were cleared by centrifugation at 31,000 x *g* for 30 min in a Sorvall RC2-B centrifuge. The supernatant extracts were recovered and used directly for the enzyme assays. For the homoserine-*O*-transsuccinylase assay, each extract was dialyzed for 8 hrs at 4° against 1000 volumes of 50 mM potassium phosphate buffer at pH 7.3.

Enzyme assays

Homoserine-O-transsuccinylase was assayed by adaptation of the homoserine transacetylase exchange assay of Nagai and Kerr

(1971), as described by Savin, Flavin, and Slaughter (1972). The assay is based on the ability of the enzyme to catalyze an exchange reaction between ^{3}H-L-homoserine and *O*-succinyl-L-homoserine. The formation of ^{3}H-*O*-succinyl-L-homoserine was followed by first converting it to ^{3}H-*N*-succinyl-L-homoserine, and then separating the latter compound from ^{3}H-L-homoserine on 0.5 x 2.5 cm Dowex-50 (H^+) (50 - 100 mesh) columns. Feedback inhibition of this enzyme by L-methionine, *S*-adenosylmethionine, and α-methyl-DL-methionine, as reported in the literature (Lee, Ravel, and Shive, 1966; Schlessinger, 1967; Savin, Flavin, and Slaughter, 1972) was demonstrable with this assay. The specific activity is expressed as nmoles ^{3}H-*O*-succinyl-L-homoserine formed in 20 min per mg protein at 37°.

Cystathionine-γ-synthetase was assayed by measuring the amount of α-ketobutyrate formed from *O*-succinyl-L-homoserine as described by Kaplan and Flavin (1966). The specific activity is expressed as the decrease in absorbance at 340 nm per 20 min per mg protein at 37°.

β-cystathionase was assayed by estimating sulfhydryl production from DL-cystathionine using the aromatic disulfide 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and measuring the amount of colored aryl mercaptan formed, as described by Flavin (1962). The specific activity is expressed as the increase in absorbance at 412 nm per min per mg protein at 22°.

N^5,N^{10}-Methylenetetrahydrofolate reductase was assayed by measurement of the menadione-dependent oxidation of ^{14}C-*N^5*-methyltetrahydrofolate to tetrahydrofolate and ^{14}C-formaldehyde, as described by Dickerman and Weissbach (1964). The specific activity is expressed as nmoles formaldehyde formed in 30 min per mg protein at 37°.

ATP:Methionine S-adenosyltransferase was assayed by a modification of the procedure described by Tabor and Tabor (1971). The formation of ^{14}C-*S*-adenosylmethionine was followed by its separation from ^{14}C-L-methionine on 0.5 x 2.5 cm BioRex-70 (50 - 100 mesh) columns, as described by Holcomb and Shapiro (1975) and modified by C. Somerville (unpublished). The specific activity is expressed as μmoles *S*-adenosylmethionine formed in 20 min per mg protein at 37°.

Protein concentration was estimated by the Folin-Ciocalteau reagent or by the Biuret reaction, as described by Layne (1957).

Leuconostoc mesenteroides P60 bioassay for methionine

Saturated cultures of *L. mesenteroides* P60 were prepared in a medium containing equal amounts of Difco methionine assay medium and Difco brain heart infusion. After 12 hrs incubation at 37°, 1 ml of the saturated culture was centrifuged, washed with 10 ml saline, and resuspended in saline to a cell density of ∿ 10^6 cells per ml. Methionine standards and the unknown samples (2.5 ml) were made up to a final volume of 5 ml with methionine assay medium. These

samples were then inoculated with one drop of *L. mesenteroides* P60 suspension in saline. After 24 hrs incubation with aeration at 37°, the turbidity of each sample was determined at 660 nm in a Zeiss PMQII spectrophotometer. Under these conditions the bioassay is sensitive to concentrations of 0.3 - 15 μg of L-methionine per assay.

Measurement of methionine pool sizes and methionine excretion

The endogenous free methionine pools were extracted essentially as described by Hobson (1974). The methionine content in 0.5 ml and 1.0 ml samples of the extracts was measured by using the *L. mesenteroides* P60 bioassay. The endogenous methionine pool is expressed as nmoles L-methionine per mg wet weight of bacteria.

Methionine excretion was determined in the following manner. Two 5 ml cultures were grown for 18 - 24 hrs at 30° in minimal medium containing the required supplements. The bacteria were removed from the medium by centrifugation at 18,000 x *g* for 10 min. The supernatant was sterilized at 121° for 7 min, cleared by centrifugation, and stored at 4°. Duplicate samples (1 ml) from each culture were assayed for methionine content by the *L. mesenteroides* P60 bioassay. The methionine excreted is expressed as nmoles L-methionine per ml ($3\text{-}4 \times 10^9$ cells) of culture medium.

RESULTS

Isolation and classification of ethionine resistant mutants

Ninety spontaneous L-ethionine resistant mutants were isolated from the strain CSH4 (F^- *lacZ*$_{am}$ *trp*$_{am}$ *strA*) and designated as AA1201 - AA1290. To differentiate methionine regulatory mutants (*metJ* or *metK*) from other nonregulatory ethionine resistant mutants (Morowicz, 1975), P1*vir* lysates grown on these mutants were used to transduce the strains B36 (*metB*) and AT2475 (*serA*) to $metB^+$ and $serA^+$, respectively. Fifty $metB^+$ and fifty $serA^+$ colonies from each cross were tested for the cotransduction of ethionine resistance. As expected, three distinct categories of ethionine resistant mutants were observed by this preliminary characterization. The first category was comprised of 45 *metJ* mutants which were closely linked with the *metB* gene, the second category consisted of 39 *metK* mutants which were cotransducible with the *serA* gene, and the third category included 6 ethionine resistant mutants that were cotransducible with neither *metB* nor *serA*. Of the ninety mutants, 6 were found to be ethionine sensitive when lysogenized with ϕ80p*su3*$^+$ and were classified as *amber* ethionine resistant mutants. These included one *amber metJ* mutant (AA1209), one *amber metK* mutant (AA1233), and 4 *amber* nonregulatory mutants (AA1281, AA1283, AA1287, and AA1290).

The metJ mutants

A total of 45 *metJ* mutants were isolated. Table 2 lists the mutants identified as *metJ* and gives the cotransduction frequencies

TABLE 2 - Mapping of *metJ* mutants

Strain	% cotransduction of ethionine resistance with *metB*	Strain	% cotransduction of ethionine resistance with *metB*
CSH4	0	AA1236	94
AA1204	92	AA1237	96
AA1205	94	AA1238	96
AA1206	94	AA1245	94
AA1208	96	AA1249	94
AA1209	98	AA1250	92
AA1211	94	AA1252	94
AA1212	94	AA1256	96
AA1213	96	AA1257	96
AA1216	96	AA1258	100
AA1217	96	AA1262	96
AA1218	94	AA1264	96
AA1219	96	AA1265	98
AA1220	94	AA1267	94
AA1221	94	AA1268	94
AA1222	94	AA1271	94
AA1223	94	AA1275	100
AA1224	96	AA1277	96
AA1227	96	AA1279	96
AA1230	96	AA1284	96
AA1231	96	AA1285	98
AA1232	96	AA1286	98
AA1234	96		
AA1235	96		

between *metB36* and the *metJ* alleles. Since previous work (Lawrence *et al.*, 1968; Ahmed, 1973) has shown that *metJ* mutants are derepressed in a noncoordinate manner for the methionine biosynthetic enzymes, 17 of these *metJ* mutants were assayed for N^5,N^{10}-methylenetetrahydrofolate reductase and β-cystathionase activities to confirm the mapping results. These enzyme assays were chosen as it was reported (Ahmed, 1973; Whitehouse and Smith, 1973) that, among the various *met* loci, *metF* and *metC* are, respectively, the most derepressed and the least derepressed loci in *metJ* mutants. As shown in Table 3, all of the *metJ* mutants assayed exhibited constitutive synthesis for both enzymes. These results clearly affirm the classification of these ethionine resistant mutants as *metJ* regulatory mutants.

The *metJ* mutant, AA1209, was found to be ethionine sensitive when lysogenized with ϕ80p$su3^+$, indicating that *metJ1209* was an *amber* mutation. To determine if the *metJ1209* mutation was suppressible by other nonsense suppressors, spontaneous su^+ derivatives of AA1209 (lac_{am} trp_{am} $metJ1209_{am}$ su^o) were isolated by selecting lac^+ trp^+ revertants simultaneously. Three revertants were classified as su^+ by their ability to support the growth of T4*am*, which propagates on sensitive su^+ strains. However, when tested for ethionine sensitivity, only one su^+ revertant (AA1209-1) exhibited slight sensitivity, whereas others were completely resistant. This was interpreted to mean that the su^+ revertants had acquired suppressor tRNAs which inserted amino acids that were incapable of restoring wild type *metJ* function. To confirm the identification of AA1209 as an *amber metJ* mutant, the specific activities of cystathionine-γ-synthetase, β-cystathionase,

TABLE 3 - Enzyme activities of selected ethionine resistant mutants derived from CSH4

Strain	Mutant class	Relative activities[a]	
		N^5,N^{10}-methylenetetrahydrofolate reductase (*metF*)	β-cystathionase (*metC*)
CSH4	wild type	1.0	1.0
AA1203	*ethR*[b]	0.9	0.8
AA1209	*metJ (amber)*	11.2	23.7
AA1233	*metK (amber)*	5.7	4.0
AA1245	*metJ*	5.9	10.3
AA1249	*metJ*	3.9	18.3
AA1250	*metJ*	6.6	14.3
AA1252	*metJ*	11.5	15.0
AA1256	*metJ*	5.0	16.4
AA1257	*metJ*	14.5	21.0
AA1258	*metJ*	15.7	23.3
AA1262	*metJ*	6.9	11.0
AA1264	*metJ*	8.7	24.0
AA1265	*metJ*	3.7	10.1
AA1267	*metJ*	4.7	9.0
AA1271	*metJ*	13.8	19.3
AA1275	*metJ*	3.3	5.1
AA1277	*metJ*	9.4	22.3
AA1279	*metJ*	6.7	29.7
AA1281	*ethR (amber)*	1.4	1.7
AA1283	*ethR (amber)*	1.0	1.3
AA1284	*metJ*	8.5	20.7
AA1287	*ethR (amber)*	1.8	1.6
AA1290	*ethR (amber)*	1.7	1.2

---

[a] The enzyme activities of the mutants are expressed relative to the specific activity of the parent strain (CSH4), which is taken as 1.0 for each enzyme. The actual specific activities of the two enzymes in strain CSH4 were: (i) N^5,N^{10}-methylenetetrahydrofolate reductase, 14.2; and (ii) β-cystathionase, 0.120. The units of specific activities are defined under "Materials and Methods."

[b] *ethR* refers to the unclassified ethionine resistant mutants which do not appear to affect methionine regulation.

and ATP:Methionine *S*-adenosyltransferase were compared in both the suppressed and nonsuppressed states. As shown in Table 4, the *metJ1209* mutation was unequivocally identified as an *amber* mutation that was efficiently suppressed by $su3^{+}$. As expected, the spontaneous su^{+} derivative, AA1209-1, did not fully restore wild type *metJ* function.

The metK mutants

The total of 39 *metK* mutants were identified. The cotransduction frequencies of the *metK* mutants with *serA* are given in Table 5. The variation in cotransduction frequencies found (18% to 40%) was also reflected in the sizes of the transductant clones, as reported by Maas (1972). Large and small transductant colonies showed cotranduction frequencies with the *serA* locus of approximately 10% to 50%, respectively.

One *metK* mutant, AA1233, is believed to be an *amber* mutation as this strain is completely sensitive to ethionine when lysogenized with $\phi 80psu3^{+}$. AA1233 was assayed in the su^{o} condition for N^5,N^{10}-methylenetetrahydrofolate reductase and β-cystathionase activities (Table 3) and the derepressed levels of activities found were characteristic of other *amber metK* mutants (Morowicz, 1975).

Unclassified ethionine resistant mutants

Six of the L-ethionine resistant mutants failed to show detectable cotransduction with *metB* or *serA*. In each case 200 transductant colonies were tested for cotransduction of ethionine resistance and none was found. As shown in Table 3, the enzyme

TABLE 4 - Enzyme activities of *amber* mutant *metJ1209* in the presence and absence of *amber* suppressors

Strain	Relative genotype	Relative activities[a]		
		Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
CSH4	$metJ^{+}$	1.0	1.0	1.0
CSH4 (ϕ80p$su3^{+}$)	$metJ^{+}$ $su3^{+}$	1.0	1.0	1.3
AA1209	*metJ1209*	12.4	6.6	2.2
AA1209 (ϕ80p$su3^{+}$)	*metJ1209* $su3^{+}$	0.9	1.1	1.5
AA1209-1	*metJ1209* su^{+}_{am}	6.6	2.8	1.7

[a] The enzyme activities of AA1209 and its su^{+} derivatives are expressed relative to the specific activity of the parent strain (CSH4), which is taken as 1.0 for each enzyme. The actual specific activities of the three enzymes in strain CSH4 were: (i) cystathionine-γ-synthetase, 0.172; (ii) β-cystathionase, 0.135; and (iii) ATP:Methionine *S*-adenosyltransferase, 11.45. The units of specific activities are defined under "Materials and Methods."

TABLE 5 - Mapping of *metK* mutants

Strain	% cotransduction of ethionine resistance with *serA*	Strain	% cotransduction of ethionine resistance with *serA*
CSH4	0	AA1251	24
AA1201	24	AA1253	22
AA1202	22	AA1254	32
AA1207	40	AA1255	30
AA1210	22	AA1259	24
AA1214	25	AA1260	28
AA1215	28	AA1261	28
AA1225	34	AA1263	18
AA1226	38	AA1266	30
AA1228	28	AA1269	28
AA1229	26	AA1270	26
AA1233	24	AA1272	18
AA1240	18	AA1273	38
AA1241	26	AA1274	30
AA1242	26	AA1276	22
AA1243	26	AA1278	32
AA1244	26	AA1280	28
AA1246	20	AA1282	30
AA1247	20	AA1288	30
AA1248	24	AA1289	20

activities of five of these mutants (AA1203, AA1281, AA1283, AA1287, and AA1290) are very similar to the wild type, which indicates that these mutants probably do not affect methionine regulation. Four of these mutants are believed to be *amber* mutations as they were ethionine sensitive when lysogenized with ϕ80p*su3*$^+$. This class of mutants has been studied previously by Morowicz (1975) who found that the majority of spontaneous DL-ethionine resistant mutants also appear to belong to this class of nonregulatory mutants. Several mutants of this class were also induced by bacteriophage Mu-1*cts62* (unpublished).

The *metK* and nonregulatory ethionine resistant mutants isolated in the course of this work were not analyzed further, since the aim of this study was to analyze the *metJ* mutants.

Orientation of metJ with respect to the flanking markers glpK, metB, and argH

Two different orientations of the *metJ* gene with respect to flanking markers have been published. Su and Greene (1971) reported the gene order *metB metJ metF*, and Ahmed (1973) suggested the gene sequence *metJ metB metF*, which is the sequence found in *S. typhimurium*. However, subsequent mapping results based on four-point crosses (Ahmed, unpublished) indicated that the sequence was *metB metJ metF*. In order to clarify this discrepancy, a transduction cross employing the closely linked markers *glpK metB metJ argH* was carried out. P1*vir* lysates of several *metJ* mutants were used to transduce the recipient strain R1 (*glpK metB argH*), and metB$^+$

transductants were selected. The transductants were scored for glpK$^+$, metJ$^-$, and argH$^+$ markers. Unfortunately, metJ$^+$ or metJ$^-$ recombinants could not be selected directly, and were scored as ethionine sensitive or resistant, respectively.

As illustrated in Figure 3, the two gene orders predict different frequencies of cotransduction of *glpK* and *argH* markers among metB$^+$ metJ$^+$ recombinants. The gene order *glpK metB metJ argH* predicts that glpK$^+$ metB$^+$ metJ$^+$ argH$^-$ transductants should occur more frequently than glpK$^-$ metB$^+$ metJ$^+$ argH$^+$ transductants. Only two crossovers are required to generate the former class, whereas four crossovers are required for the latter class. On the other hand, the alternate gene sequence, *glpK metJ metB argH*, predicts that glpK$^-$ metJ$^+$ metB$^+$ argH$^+$ transductants (which arise by two crossovers) should occur more frequently than glpK$^+$ metJ$^+$ metB$^+$ argH$^-$ transductants (which require four crossovers). The results of four crosses are presented in Table 6. It is clear that in all crosses performed, the frequency of glpk$^+$ metB$^+$ metJ$^+$ argH$^-$ transductants was greater than the frequency of glpK$^-$ metB$^+$ metJ$^+$ argH$^+$ transductants. No ambiguous or contradictory results were found with any of the *metJ* alleles tested. These results establish that the correct clockwise gene order is *glpK metB metJ argH*.

The map distances between the *glpK*, *metB*, *metJ*, and *argH* loci, based on cotransduction frequencies observed in these experiments, are presented in Figure 4. The frequencies reported were obtained by averaging the results of all crosses performed. Little variation from these frequencies was observed.

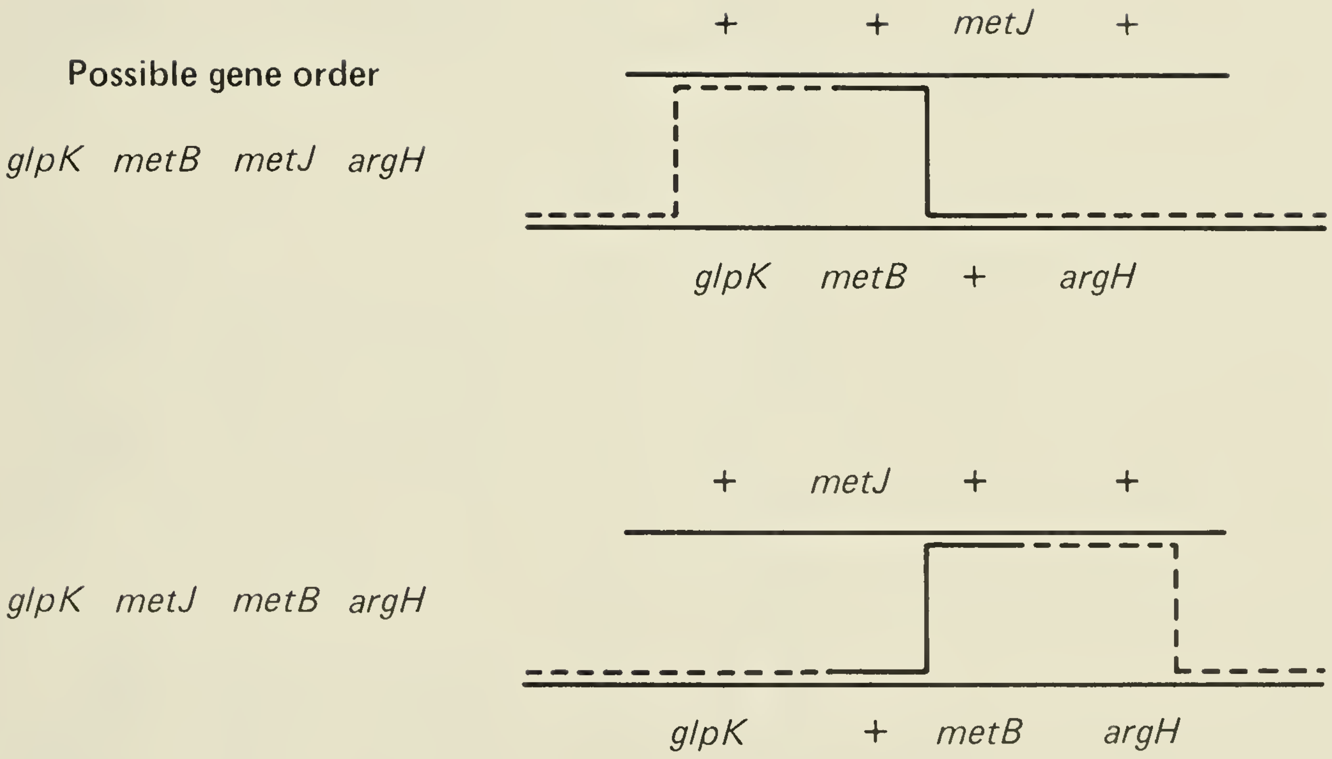

FIGURE 3 - Illustration of the four-point transduction crosses performed for the determination of the order of the *glpK*, *metB*, *metJ*, and *argH* genes. The donor fragment is represented by the top line and the recipient chromosome by the bottom line for each cross. MetB$^+$ transductants were selected and scored for the inheritance of glpK, metJ, and argH markers. The majority of the metB$^+$ metJ$^+$ transductants would be expected to be glpK$^+$ argH$^-$ if the correct order was *glpK metB metJ argH*, and glpK$^-$ argH$^+$ if the order was *glpK metJ metB argH*.

TABLE 6 - Ordering of *glpk*, *metB*, *metJ*, and *argH* genes by four-point transduction crosses

Donor	Total number of $metB^+$ transductants tested	Number scored as $metB^+$ $metJ^+$	Number scored as $glpK^+$ $metB^+$ $metJ^+$ $argH^-$	Number scored as $glpK^-$ $metB^+$ $metJ^+$ $argH^+$	Gene order inferred
metJ36	314	22 (7.0%)	7 (2.2%)	1 (0.3%)	*glpK metB metJ argH*
metJ101	297	20 (6.7%)	11 (3.7%)	1 (0.3%)	*glpK metB metJ argH*
metJ120	944	70 (7.4%)	37 (3.9%)	8 (0.8%)	*glpK metB metJ argH*
metJ148	558	30 (5.4%)	12 (2.2%)	4 (0.7%)	*glpK metB metJ argH*

P1 lysates prepared on various donor strains ($glpK^+$ $metB^+$ $metJ^-$ $argH^+$) were used to transduce the recipient strain R1 ($glpK^-$ $metB^-$ $metJ^+$ $argH^-$). $MetB^+$ transductants were selected and scored for the glpK, metJ, and argH markers.

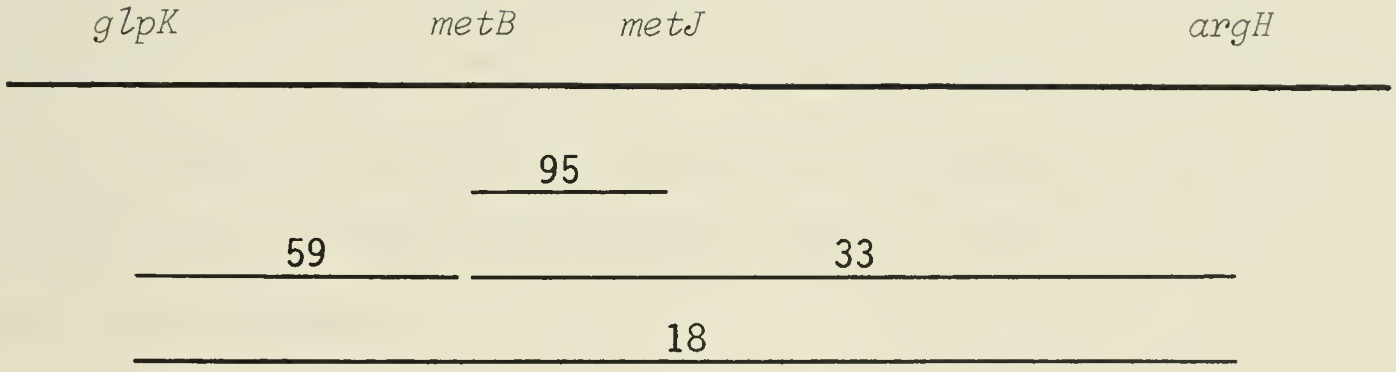

FIGURE 4 - Map of the *metJ* region of the *E. coli* chromosome showing the frequencies of cotransduction of various genes by phage P1 . The distances are not drawn to scale .

Fine structure mapping of the metJ gene

The relative order of twenty different sites of mutations within the *metJ* gene with respect to the outside markers, *glpK* and *argH*, was determined by using four-point crosses. A series of glpK$^-$ argH$^-$ derivatives of each of the twenty *metJ* alleles was constructed in the following manner. Sixteen *metJ* alleles, which were not suppressible by *amber* suppressors, were crossed into strain R1, and the remaining four *amber metJ* alleles were transduced into strain R2 by phage P1. MetB$^+$ transductants were selected, and a series of *glpK metJ argH* recombinants for each *metJ* allele were isolated. As expected, these derivatives were found to be derepressed for the enzymes cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase, which is characteristic of *metJ* mutants (Table 7).

For fine structure analysis, transduction crosses of the type glpK$^-$ metJ1$^-$ argH$^-$ x glpK$^+$ metJ2$^-$ argH$^+$ were carried out. In each case, the recipient was glpK$^-$ metJ$^-$ argH$^-$ and the donor was glpK$^+$ metJ$^-$ argH$^+$. GlpK$^+$ or argH$^+$ transductants were selected and scored for metJ$^+$ recombinants and the outside marker (*argH* or *glpK*, depending upon the selected marker). The different *metJ* alleles were crossed in all reciprocal combinations so that each allele was employed as the donor in one cross, and as recipient in the other.

The crossover events required between the *metJ1* and *metJ2* mutation sites to produce metJ$^+$ recombinants and their relationship to the outside markers *glpK* and *argH* are presented in Figures 5 and 6.

TABLE 7 - Enzyme activities of *metJ* derivatives of R1 (GH306 - GH384) and R2 (GH390 - GH398)

Strain	*metJ* allele	Relative activities[a]		
		Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
GH311	*metJ*$^+$	1.0	1.0	1.0
GH350	*metJ36*	18.0	6.3	1.9
GH330	*metJ101*	17.9	6.5	1.8
GH306	*metJ120*	21.4	7.7	1.9
GH320	*metJ148*	18.1	8.1	2.1
GH361	*metJ1245*	11.6	4.5	1.8
GH362	*metJ1249*	14.8	10.7	2.2
GH364	*metJ1252*	16.9	15.0	1.8
GH366	*metJ1256*	17.0	12.2	1.7
GH369	*metJ1257*	15.5	10.3	1.9
GH371	*metJ1258*	17.3	14.5	1.8
GH375	*metJ1264*	16.3	6.6	2.0
GH377	*metJ1271*	17.5	12.4	1.9
GH378	*metJ1275*	11.3	5.5	2.1
GH380	*metJ1277*	18.4	10.4	1.6
GH383	*metJ1279*	19.1	10.8	2.0
GH384	*metJ1284*	16.6	6.8	2.1
GH398	*metJ*$^+$	1.0	1.0	1.0
GH396	*metJ65*$_{am}$	20.9	12.1	2.1
GH392	*metJ89*$_{am}$	18.5	9.0	1.8
GH394	*metJ93*$_{am}$	19.5	10.8	2.3
GH390	*metJ1209*$_{am}$	19.0	7.2	2.1

---

[a] The enzyme activities of the *metJ* mutants are expressed relative to the specific activities of the parent strains, R1 (GH311) and R2 (GH398), which are taken as 1.0 for each enzyme. The actual specific activities for the enzymes cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase were: 0.200, 0.122, and 14.47, respectively, for strain R1 (GH311); and 0.172, 0.118, and 13.56, respectively, for strain R2 (GH398). The units of specific activities are defined under "Materials and Methods."

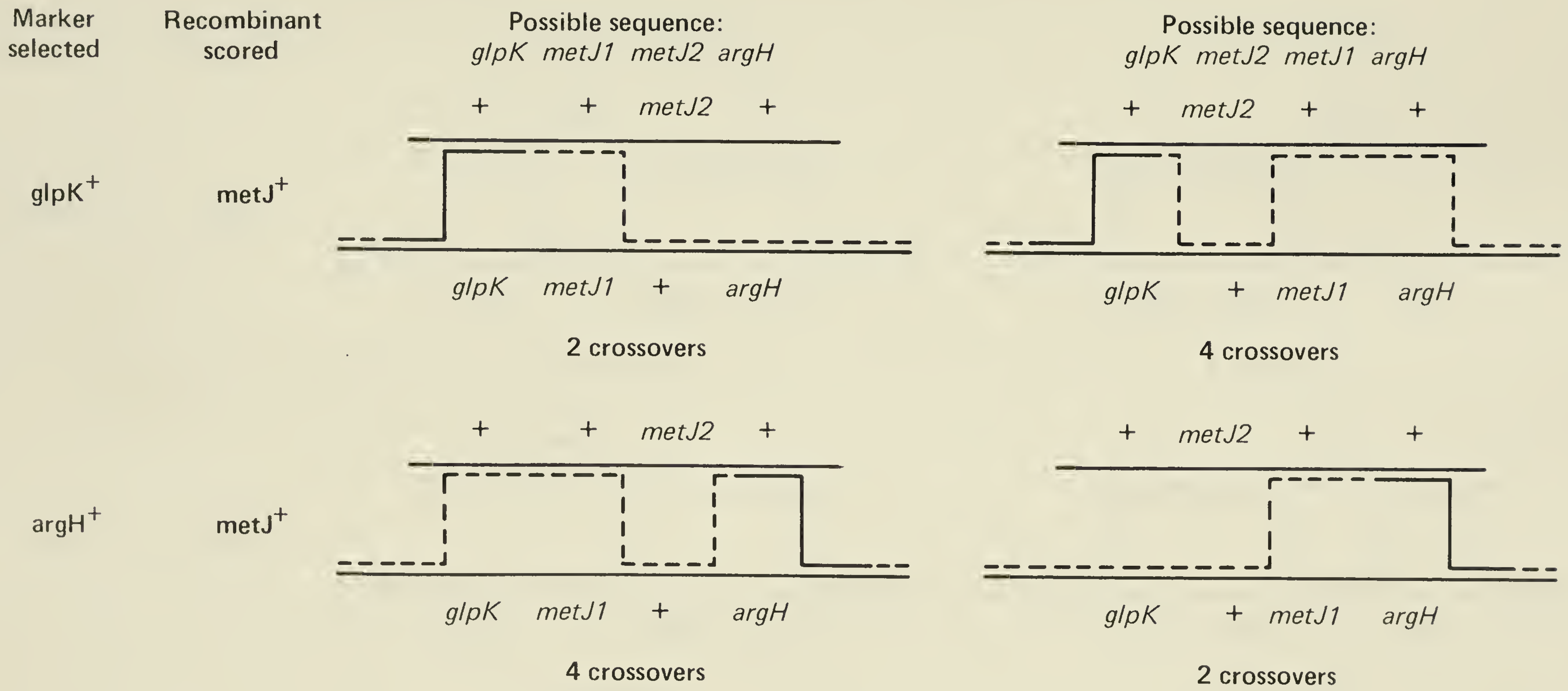

FIGURE 5 - CROSS I . Illustration of the four-point transduction crosses performed for the determination of the order of different *metJ* mutations. For each cross, the donor fragment (shown here to carry the *metJ2* mutation) is represented by the upper line, and the recipient chromosome (carrying the *metJ1* mutation) is shown by the lower line. The number of crossovers required to generate metJ$^+$ recombinants when glpK$^+$ or argH$^+$ transductants are selected is indicated below each cross. The two possible sequences of the *metJ1* and *metJ2* mutations (with respect to the outside markers) predict different frequencies of metJ$^+$ recombinants and different frequencies of cotransduction of the distal unselected marker among metJ$^+$ recombinants. The reciprocal cross is illustrated in Figure 6.

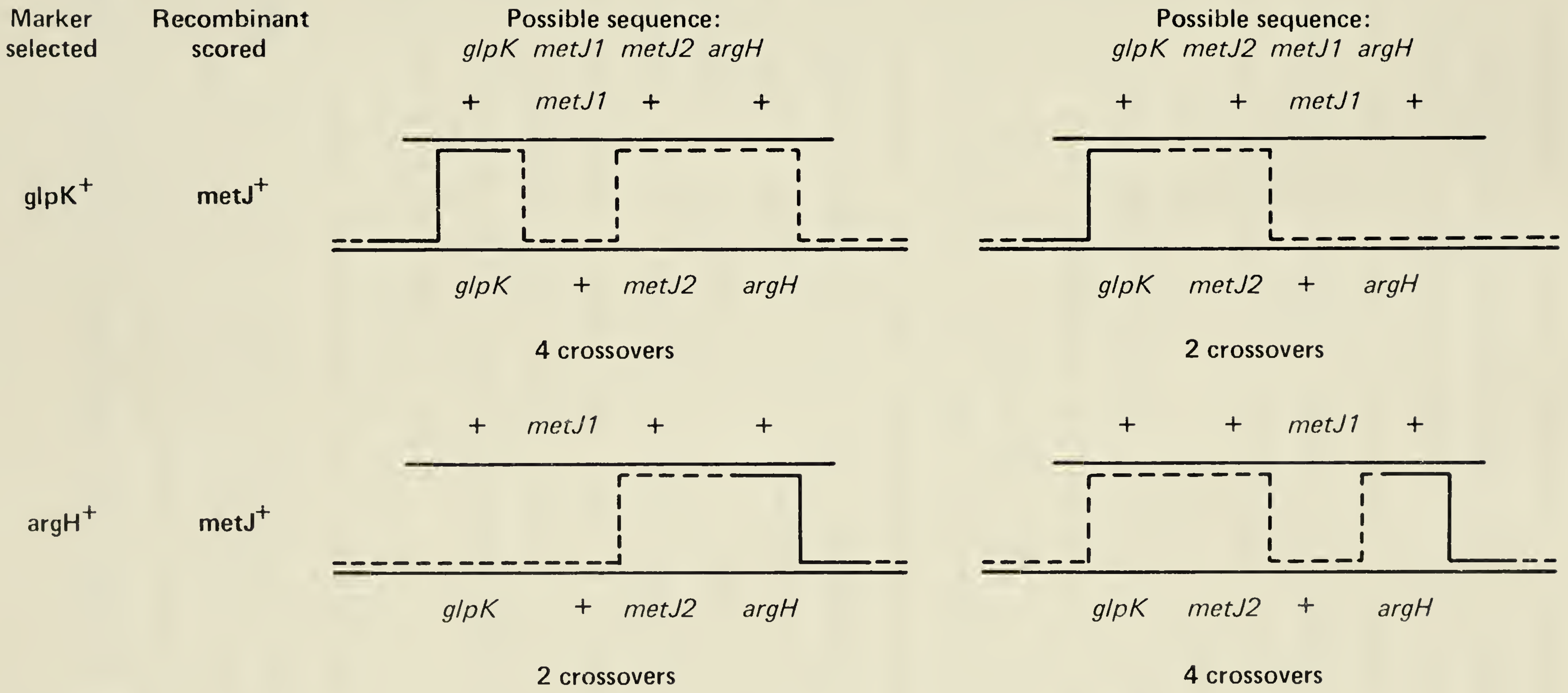

FIGURE 6 - CROSS II . Illustration of four-point crosses, performed in a manner reciprocal to those depicted in Figure 5, for the purpose of ordering various *metJ* mutations. The donor fragment is represented by the upper line and the recipient chromosome by the lower line for each cross. In these crosses, *metJ1* is the donor and *metJ2* is the recipient. GlpK$^+$ or argH$^+$ transductants are selected and the number of crossovers required to generate metJ$^+$ recombinants among the transductants is indicated below each cross. The two possible orientations of the *metJ* mutations predict different frequencies of metJ$^+$ recombinants and different frequencies of cotransduction of the distal unselected marker among metJ$^+$ recombinants.

It is obvious that the distribution of *glpK* and *argH* markers among the metJ$^+$ recombinants in the two crosses would allow unambiguous ordering of the sites of the two *metJ* mutations. As shown in Figure 5 (CROSS I), if the correct sequence is *glpK metJ1 metJ2 argH,* two crossovers would be necessary to give metJ$^+$ recombinants if glpK$^+$ transductants are selected. Moreover, the frequency of *glpK*$^+$ *metJ*$^+$ *argH*$^-$ class of recombinants would be expected to be higher than the *glpK*$^+$ *metJ*$^+$ *argH*$^+$ class. If the selected marker is argH$^+$, then four crossovers would be required to give metJ$^+$ recombinants, and *glpK*$^+$ *metJ*$^+$ *argH*$^+$ recombinants would be found in excess over the *glpK*$^-$ *metJ*$^+$ *argH*$^+$ class. On the other hand, if the correct sequence is *glpK metJ2 metJ1 argH,* then the situation would be exactly the opposite. When *glpK*$^+$ transductants are selected, four crossovers would be required to give metJ$^+$ recombinants, and the *glpK*$^+$ *metJ*$^+$ *argH*$^+$ recombinants would be the majority class and *glpK*$^+$ *metJ*$^+$ *argH*$^-$ recombinants would be the minority class. If the selected marker is argH$^+$, then two crossovers would be required to give metJ$^+$ recombinants, and *glpK*$^-$ *metJ*$^+$ *argH*$^+$ transductants would constitute the majority class. In the reciprocal cross (Figure 6, CROSS II), the entire situation would be reversed and the results would simply confirm the results of cross I.

The results of four-point crosses obtained with three *metJ* alleles, viz., *metJ101, metJ120,* and *metJ148,* according to the scheme outlined in Figures 5 and 6, are presented in Table 8. It is clear that (i) depending upon the marker selected (*glpK* or *argH*), the genotypes of the *metJ*$^+$ recombinants with respect to outside markers

TABLE 8 - Ordering of *metJ101*, *metJ120*, and *metJ148* alleles by reciprocal four-point transduction crosses

Donor[a]	Recipient[b]	Selected marker	Number of transductants tested	Number of metJ$^+$ recombinants found	Distribution of outside markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ101	*metJ120*	glpK$^+$	240	1	1		0	*glpK metJ120 metJ101 argH*
metJ101	*metJ120*	argH$^+$	280	1		0	1	" " " "
metJ120	*metJ101*	glpK$^+$	210	3	0		3	" " " "
metJ120	*metJ101*	argH$^+$	248	7		7	0	" " " "
metJ101	*metJ148*	glpK$^+$	286	8	8		0	*glpK metJ148 metJ101 argH*
metJ101	*metJ148*	argH$^+$	280	10		1	9	" " " "
metJ148	*metJ101*	glpK$^+$	208	2	0		2	" " " "
metJ148	*metJ101*	argH$^+$	248	12		10	2	" " " "
metJ120	*metJ148*	glpK$^+$	242	11	10		1	*glpK metJ148 metJ120 argH*
metJ120	*metJ148*	argH$^+$	248	2		0	2	" " " "
metJ148	*metJ120*	glpK$^+$	216	3	0		3	" " " "
metJ148	*metJ120*	argH$^+$	248	13		10	3	" " " "

[a] The donor in each cross was glpK$^+$ metJ$^-$ argH$^+$.

[b] The recipient in each cross was glpK$^-$ metJ$^-$ argH$^-$.

were different, and (ii) the frequencies of metJ$^+$ recombinants found in each reciprocal cross were consistently different. Taken together, these results establish the order *glpK metJ148 metJ120 metJ101 argH*. The twenty different *metJ* alleles used in this study gave similar unambiguous results in reciprocal crosses in various combinations. The results are summarized in Appendix I and the sequence of mutational sites within the *metJ* gene as inferred from these results is presented in Figure 7. It is interesting to note that three *amber metJ* mutations form a cluster toward the right end of the map. The possible significance of this is discussed later.

Dominant metJ alleles

Most of the *metJ* mutants previously isolated have been found to be recessive to the wild type. The known exceptions are one *metJ* mutant of *S. typhimurium* reported by Chater (1970) and one *amber metJ* mutant of *E. coli* described by Morowicz (1975) from this laboratory, which appeared to be dominant *metJ* mutants. In the partial diploid state together with *metJ*$^+$, these mutants continued to exhibit ethionine resistance and constitutive enzyme synthesis.

In order to determine dominance of the *metJ* mutants of *E. coli* included in this study, *metJ*$^-$/*metJ*$^+$ merodiploids were constructed by transferring F14 bearing various *metJ* alleles into GH311 (F$^-$ *ilv glpK metJ*$^+$ *argH su*$^+_{\text{am}}$) and GH398 (F$^-$ *ilv glpK metJ*$^+$ *argH su*$^o_{\text{am}}$), or F14 *metJ*$^+$ into *metJ* derivatives of R1 (F$^-$ *ilv glpK metJ*$^-$ *argH su*$^+_{\text{am}}$) and R2 (F$^-$ *ilv glpK metJ*$^-$ *argH su*$^o_{\text{am}}$). The F-prime F14 was chosen as the donor episome because the markers

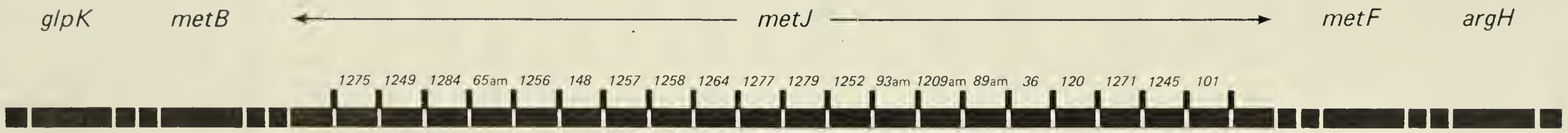

FIGURE 7 - The order of mutational sites within the *metJ* gene which codes for the repressor for methionine biosynthesis in *E. coli*. The linear order of twenty *metJ* alleles with respect to the *glpK* and *argH* loci was determined by reciprocal four-point transduction crosses. The distances are not drawn to scale.

ilv$^+$, *glpK*$^+$, and *argH*$^+$ borne by this episome can be used for the selection of *metJ*$^-$/*metJ*$^+$ merodiploids. The different *metJ* alleles were transduced onto F14 by selecting for metB$^+$ transductants of a *metB* derivative (GH418) of the original F14 strain, AB1206. Since the strain GH418 carries an *amber* suppressor, the transduction of only nonsuppressible *metJ* alleles was detectable. The enzyme activities of various *metJ* derivatives of the F14 strain GH418 are presented in Table 9. It is evident that all of these *metJ* derivatives show constitutive synthesis of the enzymes specified by the *metB*, *metC*, and *metK* genes like the original *metJ* mutations. It was, therefore, concluded that the 16 *metJ* derivatives of GH418 had inherited the same *metJ* alleles which were present in the *metJ* derivatives of R1. The F14 metJ$^+$ allele used throughout was a metB$^+$ metJ$^+$ transductant, GH420 (F14/Δ(*ilv glpK metBJF arg*)*su*$^+_{\mathrm{am}}$), of GH418 (*metB*) to rule out effects of any unseen secondary mutations that could have occurred during mutagenesis of the episome for the isolation of the *metB* derivative.

The *metJ*$^-$/*metJ*$^+$ merodiploids were constructed by spot matings and tested for ethionine resistance. As shown in Table 10, three of the *metJ* alleles tested (viz., *metJ65*$_{\mathrm{am}}$, *metJ1271*, and *metJ1275*) were dominant over *metJ*$^+$ (i.e., these *metJ*$^-$/*metJ*$^+$ merodiploids were ethionine resistant). Of these, *metJ1271* and *metJ1275* were dominant only when located on the episome. The enzyme activities of these partial diploids and the wild type strain are compared in Table 11. When *metJ1271* and *metJ1275* alleles were present on the episome, enzyme synthesis was constitutive, indicating that the

TABLE 9 - Enzyme activities of various *metJ* derivatives of F14 strain GH418

Strain	*metJ* allele present	Relative activities[a]		
		Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
GH420	*metJ*$^+$	1.0	1.0	1.0
GH436	*metJ36*	13.2	8.5	1.8
GH431	*metJ101*	12.0	7.7	1.6
GH430	*metJ120*	11.6	8.2	1.6
GH438	*metJ148*	6.6	6.5	1.7
GH445	*metJ1245*	6.5	3.4	1.8
GH449	*metJ1249*	8.6	8.1	1.7
GH452	*metJ1252*	10.2	7.9	1.5
GH456	*metJ1256*	12.9	11.6	1.8
GH457	*metJ1257*	13.3	9.2	1.9
GH458	*metJ1258*	12.1	10.3	1.8
GH464	*metJ1264*	10.4	6.2	1.7
GH471	*metJ1271*	3.2	6.1	1.6
GH475	*metJ1275*	5.8	4.5	1.5
GH477	*metJ1277*	3.8	6.0	1.6
GH479	*metJ1279*	11.3	10.2	1.8
GH484	*metJ1284*	12.5	11.6	2.0

[a] For each enzyme, the activity is expressed relative to the specific activity of the wild type which is taken as 1.0. The actual specific activities of the wild type strain were: cystathionine-γ-synthetase, 0.232; β-cystathionase, 0.128; and ATP:Methionine *S*-adenosyltransferase, 10.02. The units of specific activities are defined under "Materials and Methods."

TABLE 10 - Dominance of *metJ* alleles in *metJ*$^{+}$/*metJ*$^{-}$ merodiploids[a]

Episomal *metJ* allele	Chromosomal *metJ* allele	Ethionine resistance	Episomal *metJ* allele	Chromosomal *metJ* allele	Ethionine resistance
metJ^{+}	*metJ*$^{+}$	-	*metJ*$^{+}$	*metJ*$^{+}$	-
metJ^{+}	*metJ1275*	-	*metJ1275*	*metJ*$^{+}$	+
metJ^{+}	*metJ1249*	-	*metJ1249*	*metJ*$^{+}$	-
metJ^{+}	*metJ1284*	-	*metJ1283*	*metJ*$^{+}$	-
metJ^{+}	*metJ1256*	-	*metJ1256*	*metJ*$^{+}$	-
metJ^{+}	*metJ148*	-	*metJ148*	*metJ*$^{+}$	-
metJ^{+}	*metJ1257*	-	*metJ1257*	*metJ*$^{+}$	-
metJ^{+}	*metJ1258*	-	*metJ1258*	*metJ*$^{+}$	-
metJ^{+}	*metJ1264*	-	*metJ1264*	*metJ*$^{+}$	-
metJ^{+}	*metJ1277*	-	*metJ1277*	*metJ*$^{+}$	-
metJ^{+}	*metJ1279*	-	*metJ1279*	*metJ*$^{+}$	-
metJ^{+}	*metJ1251*	-	*metJ1252*	*metJ*$^{+}$	-
metJ^{+}	*metJ36*	-	*metJ36*	*metJ*$^{+}$	-
metJ^{+}	*metJ120*	-	*metJ120*	*metJ*$^{+}$	-
metJ^{+}	*metJ1271*	-	*metJ1271*	*metJ*$^{+}$	+
metJ^{+}	*metJ1245*	-	*metJ1245*	*metJ*$^{+}$	-
metJ^{+}	*metJ101*	-	*metJ101*	*metJ*$^{+}$	-
metJ^{+}	*metJ*$^{+}$[b]	-			
metJ^{+}	*metJ65*$_{am}$	+			
metJ^{+}	*metJ93*$_{am}$	-			
metJ^{+}	*metJ1209*$_{am}$	-			
metJ^{+}	*metJ89*$_{am}$	-			

---

[a] The *metJ*$^{+}$/*metJ*$^{-}$ merodiploids were constructed by transferring F14 *metJ*$^{+}$ into F^{-} recipients bearing the *metJ*$^{+}$ allele or other *metJ*$^{-}$ alleles, or by transferring F14 bearing various *metJ*$^{-}$ alleles into the F^{-} recipients GH311 or GH398 which carry the *metJ*$^{+}$ allele. A negative response (-) indicates ethionine sensitivity (i.e., dominance of *metJ*$^{+}$ over *metJ*$^{-}$ in the merodiploid) and a positive response (+) indicates ethionine resistance (i.e., dominance of *metJ*$^{-}$ over *metJ*$^{+}$).

[b] The recipient strains were *su*^{o}. The reciprocal merodiploids (F14 *metJ*$_{am}$/*metJ*$^{+}$) were not constructed since the F14 donor strain was *su*$^{+}$.

TABLE 11 - Enzyme activities of *metJ* alleles exhibiting dominance in *metJ*$^{+}$/*metJ*$^{-}$ partial diploids

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]			
		Homoserine-*O*-transsuccinylase (*metA*)	Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
metJ^{+}	*metJ*$^{+}$[b]	1.0	1.0	1.0	1.0
metJ1271	*metJ*$^{+}$	5.0	4.2	5.2	1.9
metJ^{+}	*metJ1271*	-	1.0	1.7	1.4
metJ1275	*metJ*$^{+}$	-	5.3	3.2	1.9
metJ^{+}	*metJ1275*	-	0.9	1.8	1.3
metJ^{+}	*metJ*$^{+}$[c]	1.0	1.0	1.0	1.0
metJ^{+}	*metJ65*$_{am}$	4.6	5.1	6.1	2.3

[a] The activity of each enzyme is expressed relative to the specific activity of the wild type which is taken as 1.0. The actual specific activities of the wild type are given below.

[b] The recipient strains were *su*$^{+}$. Actual specific activities of the *metJ*$^{+}$/*metJ*$^{+}$ *su*$^{+}$ wild type were: homoserine-*O*-transsuccinylase, 591.1; cystathionine-γ-synthetase, 0.283; β-cystathionase, 0.102; and ATP:Methionine *S*-adenosyltransferase, 11.39.

[c] The recipient strains were *su*^{o}. Actual specific activities of the *metJ*$^{+}$/*metJ*$^{+}$ *su*^{o} wild type were: homoserine-*O*-transsuccinylase, 160.4; cystathionine-γ-synthetase, 0.618; β-cystathionase, 0.106; and ATP:Methionine *S*-adenosyltransferase, 11.87. The reciprocal merodiploid F14 *metJ65*$_{am}$/*metJ*$^{+}$ was not constructed and analyzed.

met repressor was partially defective in these *metJ*$^-$/*metJ*$^+$ merodiploids. The extents of derepression found in these *metJ*$^-$/*metJ*$^+$ merodiploids were, however, not as high as that found in the haploid *metJ* strains (Table 7). In contrast, the same *metJ* alleles acted as recessive when they were present on the chromosome and the *metJ*$^+$ allele was located on the episome. As shown in Table 11, such merodiploids exhibited wild type levels of activity indicating that a functional repressor was being produced.

In order to confirm the results presented above, the excretion of methionine by the *metJ*$^-$/*metJ*$^+$ merodiploids was determined by the methionine bioassay method. As shown in Table 12, the presence of *metJ1271* or *metJ1275* on the episome results in a three- to four-fold increase in the amount of methionine excreted into the medium as compared to the wild type. This is not unexpected for cells that synthesize the methionine biosynthetic enzymes in a constitutive manner. However, when these alleles are located on the chromosome, methionine excretion is no greater than the wild type. It would appear that *metJ1271* and *metJ1275* mutants exhibit transdominance over *metJ*$^+$ by subunit aggregation to produce nonfunctional repressors.

The dominance of the *amber* mutant *metJ65*$_{\mathrm{am}}$ over the wild type as indicated by the ethionine resistance and constitutive enzyme synthesis in F14 *metJ*$^+$/*metJ65*$_{\mathrm{am}}$ merodiploids has been reported earlier (Morowicz, 1975). The overall production of methionine in these merodiploids was also compared to the wild type. As shown in Table 12, the endogenous free methionine pool as well as the quantity of

TABLE 12 - Methionine production in *metJ*$^+$/*metJ*$^-$ merodiploids

Strain	Relative methionine production[a]	
	Intracellular pool size	Excretion into the medium
A. Chromosome *su*$^+$:		
F14 *metJ*$^+$/*metJ*$^+$	-	1.0[b]
F14 *metJ1271*/*metJ*$^+$	-	3.2
F14 *metJ*$^+$/*metJ1271*	-	0.9
F14 *metJ1275*/*metJ*$^+$	-	3.6
F14 *metJ*$^+$/*metJ1275*	-	1.0
B. Chromosome *su*^{o}:		
F14 *metJ*$^+$/*metJ*$^+$	1.0[c]	1.0[d]
F14 *metJ*$^+$/*metJ65*$_{am}$[e]	2.9	3.5

---

[a] The endogenous methionine pool sizes and methionine excretion are expressed relative to the wild type levels, which are taken as 1.0. The actual values for the wild type are given below.

[b] 0.074 nmoles methionine excreted per ml (3-4 x 10^9 cells) of culture medium.

[c] 0.066 nmoles methionine per mg wet weight of bacteria.

[d] 0.067 nmoles methionine excreted per ml (3-4 x 10^9 cells) of culture medium.

[e] The reciprocal merodiploid (F14 *metJ65*$_{am}$/ *metJ*$^+$) was not constructed and analyzed.

methionine excreted into the medium in F14 *metJ*$^{+}$/*metJ65*$_{am}$ were nearly three times higher than the wild type. These results provide additional evidence that the synthesis of methionine in F14 *metJ*$^{+}$/*metJ65*$_{am}$ occurs in an uncontrolled manner. The dominance of the *metJ65*$_{am}$ allele over the *metJ*$^{+}$ allele is most likely due to the aggregation of a unique amber fragment, or a restart fragment, with the wild type protomer giving rise to a nonfunctional oligomeric repressor.

Intra-cistronic complementation between metJ mutants

In order to study intra-cistronic complementation, *metJ*/*metJ* merodiploids were constructed by transferring F14 bearing various *metJ* alleles into F^{-} recipient strains harboring the same, or other *metJ* alleles. The relevant genotype of the F^{-} strains, which were derived from either R1 or R2, was F^{-} *ilv glpK metJ argH*. The characterization of these *metJ* recipient strains (GH300 series) has been described earlier (under "Fine structure mapping"). The F-prime F14 carries the markers ilv^{+}, glpK^{+}, and argH^{+} on the episome which can be used for the selection of *metJ*/*metJ* merodiploids. The characterization of the F14 *metJ* strains (GH400 series) has also been described earlier (under "Dominant *metJ* alleles").

The *metJ*/*metJ* merodiploids were constructed by spot matings. The F-prime strains carrying the 16 non-*amber metJ* alleles were mated in all possible pairwise combinations with F^{-} recipients carrying the same *metJ* alleles in addition to 4 *amber metJ* alleles present in F^{-} *su*^{o} background. The occurrence of complementation in the merodiploids

(which were genotypically *metJ/metJ*) was determined by observing their phenotypes on minimal plates containing ethionine. Restoration of ethionine sensitivity in the merodiploids would indicate positive complementation, whereas ethionine resistance would indicate absence of complementation.

The complementation pattern based on the restoration of ethionine sensitivity, or failure to do so, is presented in Table 13. The results are suggestive of typical intra-cistronic complementation observed between different mutants within one gene. No evidence for polarity or separate cistrons was detected.

The enzyme activities of several *metJ/metJ* merodiploids showing complementation are compared with the wild type in Table 14. It can be seen that the levels of the methionine biosynthetic enzymes in these merodiploids are close to the wild type levels, indicating that the *met* repressor was functional in these merodiploids. Elevated enzyme levels could be detected in some combinations (such as *metJ36/metJ120*, *metJ1256/metJ120*, etc.) but this amount of derepression is apparently not sufficient to confer ethionine resistance. To confirm these results the overall production of methionine in two representative merodiploids (F14 *metJ1279/metJ1284* and F14 *metJ36/metJ1279*) was also determined. As shown in Table 15, the intracellular pools of methionine as well as the relative amounts of methionine excreted into the medium by these merodiploids were comparable to the wild type. All of these results indicate restoration (or partial restoration) of normal repression.

TABLE 13 - Intra-cistronic complementation in *metJ/metJ* merodiploids[a]

Chromosomal *metJ* alleles	F14 episomal *metJ* alleles															
	1275	*1249*	*1284*	*1256*	*148*	*1257*	*1258*	*1264*	*1277*	*1279*	*1252*	*36*	*120*	*1271*	*1245*	*101*
1275	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
1284	-	-	-	-	+	+	+	-	+	+	-	-	+	-	+	+
65_{am}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1256	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
148	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
1257	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
1258	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1264	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-
1277	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1279	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	-
1252	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93_{am}	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
1209_{am}	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	-
89_{am}	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-
36	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+
120	-	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-
1271	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1245	-	-	+	+	-	-	-	+	-	+	-	+	+	-	-	+
101	-	+	+	+	-	-	-	-	-	-	-	+	-	-	+	-

---

[a] The *metJ/metJ* merodiploids were constructed by transferring F14 carrying *metJ* alleles into F^- recipients having the same, or other, *metJ* alleles. A positive response (i.e., occurrence of complementation) was indicated by the ethionine sensitivity of the merodiploid, whereas a negative response (i.e., lack of complementation) was indicated by the ethionine resistance of the merodiploid. The mutants are arranged (left to right, and top to bottom) according to their linear order along the *metJ* gene determined by fine structure mapping.

TABLE 14 - Enzyme activities of complementing *metJ/metJ* merodiploids showing restoration of normal repression

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]			
		Homoserine-*O*-transsuccinylase (*metA*)	Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
metJ$^+$	*metJ*$^+$	1.0	1.0	1.0	1.0
metJ1279	*metJ1284*	1.2	1.1	1.3	1.3
metJ1284	*metJ1279*	1.1	1.0	1.4	1.1
metJ120	*metJ1284*	-	1.5	1.6	1.7
metJ1284	*metJ120*	-	1.4	1.4	1.6
metJ120	*metJ36*	-	0.8	1.7	1.3
metJ36	*metJ120*	-	3.2	2.5	1.4
metJ1284	*metJ1257*	-	1.1	1.7	1.3
metJ1257	*metJ1284*	-	2.4	3.2	1.0
metJ1256	*metJ101*	-	1.7	2.2	1.8
metJ101	*metJ1256*	-	2.4	2.6	1.2
metJ1258	*metJ1284*	-	1.1	1.5	1.1
metJ1284	*metJ1258*	-	3.2	2.5	1.5
metJ36	*metJ1279*	0.6	1.1	2.3	1.1
metJ1279	*metJ36*	-	3.2	2.9	1.4
metJ120	*metJ1264*	-	2.0	1.3	1.7
metJ1264	*metJ120*	-	3.6	2.8	2.0
metJ1245	*metJ1284*	-	2.3	2.4	1.5
metJ1284	*metJ1245*	-	3.6	2.6	2.0
metJ120	*metJ1256*	-	1.6	2.2	1.6
metJ1256	*metJ120*	-	4.3	3.1	1.8

---

[a] The activity of each enzyme is expressed relative to the specific activity of the wild type which is taken as 1.0. The actual specific activities of the wild type were: homoserine-*O*-transsuccinylase, 531.7; cystathionine-γ-synthetase, 0.283; β-cystathionase, 0.102; and ATP:Methionine *S*-adenosyltransferase, 11.39. The units of specific activities are defined under "Materials and Methods."

TABLE 15 - Methionine production in complementing *metJ/metJ* merodiploids which exhibit restoration of normal repression

Strain	Relative methionine production[a]	
	Intracellular pool size	Excretion into the medium
F14 $metJ^+/metJ^+$	1.0[b]	1.0[c]
F14 *metJ1279/metJ1284*	1.0	0.9
F14 *metJ36/metJ1279*	0.8	1.1

[a] The endogenous methionine pool sizes and methionine excretion are expressed relative to the wild type levels, which are taken as 1.0. The actual values for the wild type are given below.

[b] 0.071 nmoles methionine per mg wet weight of bacteria.

[c] 0.074 nmoles methionine excreted per ml (3-4 x 10^9 cells) of culture medium.

An interesting observation was the complementation of three *amber metJ* alleles (viz., *metJ89*$_{am}$, *metJ93*$_{am}$, and *metJ1209*$_{am}$) with several non-*amber metJ* alleles (Table 13). These *amber metJ* alleles form a distinct cluster toward the right on the genetic map (Figure 7). Unlike the dominant *metJ65*$_{am}$ mutant (which maps toward the left end), these three *amber* mutants are recessive to the wild type (Table 10). Table 16 shows the enzyme activities of several merodiploid strains in which these *amber metJ* alleles were combined with $metJ^+$ or with some complementing $metJ^-$ alleles. The enzyme activities of $metJ^+/metJ^-_{am}$ merodiploids show that these $metJ_{am}$ alleles are truly recessive to the wild type. The activities of the $metJ^-/metJ^-_{am}$ merodiploids indicate that normal *met* repression was restored in these merodiploids. These results suggest that either the prematurely terminated fragment, or a restart fragment from these $metJ_{am}$ mutants is capable of aggregating with other mutant protomers, and the resultant oligomeric *met* repressor has functional capacity.

The complementation pattern of the *metJ* mutants, as inferred by restoration of ethionine sensitivity (Table 13), is reproduced in Table 17 together with results on repression of the *metA, metB, metC,* and *metK* enzymes (Table 14). These summarized results show that several complementing *metJ/metJ* and $metJ/metJ_{am}$ merodiploids have enzyme levels comparable to the wild type, which should account for the restoration of ethionine sensitivity, and indicates that different mutationally altered *metJ* protomers are capable of aggregating to form a functional, or a semi-functional, repressor.

TABLE 16 - Enzyme activities of merodiploids containing $metJ_{am}$ mutations to show their recessiveness to the wild type and their ability to complement other *metJ* mutations

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]		
		Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
$metJ^{+}$	$metJ^{+}$	1.0	1.0	1.0
$metJ^{+}$	$metJ89_{am}$	1.0	1.0	1.0
$metJ^{+}$	$metJ93_{am}$	1.0	1.3	1.2
$metJ^{+}$	$metJ1209_{am}$	0.9	0.9	1.1
metJ36	$metJ89_{am}$	1.4	1.3	1.0
metJ1252	$metJ89_{am}$	0.8	1.0	0.9
metJ1258	$metJ89_{am}$	1.2	1.3	1.2
metJ1279	$metJ89_{am}$	0.8	1.2	1.1
metJ1279	$metJ93_{am}$	1.2	1.6	1.0
metJ120	$metJ1209_{am}$	2.3	1.7	1.5
metJ1284	$metJ1209_{am}$	1.4	1.6	1.4

---

[a] The activity of each enzyme is expressed relative to the specific activity of the wild type which is taken as 1.0. The actual specific activities of the wild type were: cystathionine-γ-synthetase, 0.175; β-cystathionase, 0.112; and ATP:Methionine *S*-adenosyltransferase, 10.76. The units of specific activities are defined under "Materials and Methods."

TABLE 17 - Intra-cistronic complementation between *metJ* mutants which results in restoration of wild type regulatory behavior[a]

Chromosomal *metJ* alleles	F14 episomal *metJ* alleles															
	1275	*1249*	*1284*	*1256*	*148*	*1257*	*1258*	*1264*	*1277*	*1279*	*1252*	*36*	*120*	*1271*	*1245*	*101*
1275	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
1284	-	-	-	-	+	⊞	⊕	-	+	⊕	-	-	⊕	-	⊕	+
*65*am	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1256	-	-	-	-	-	-	-	-	-	-	-	-	⊕	-	+	⊞
148	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
1257	-	-	⊕	-	-	-	-	-	-	+	-	-	-	-	-	-
1258	-	-	⊞	-	-	-	-	-	-	-	-	-	-	-	-	-
1264	-	-	-	-	+	-	-	-	-	-	-	-	⊕	-	+	-
1277	-	-	⊕	-	-	-	-	-	-	-	-	-	-	-	-	-
1279	-	-	⊕	-	-	⊕	-	-	-	-	-	⊕	-	-	+	-
1252	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*93*am	-	-	-	-	-	-	-	-	-	⊕	-	-	-	-	-	-
*1209*am	+	+	⊕	-	+	-	-	+	+	+	-	-	⊕	+	+	-
*89*am	-	-	-	-	-	-	⊕	-	-	⊕	⊕	⊕	-	-	-	-
36	-	-	-	-	-	-	-	-	-	⊞	-	-	⊕	-	+	+
120	-	-	⊕	⊞	-	-	-	⊞	-	-	-	⊞	-	-	+	-
1271	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1245	-	-	⊞	+	-	-	-	+	-	+	-	+	+	-	-	+
101	-	+	+	⊕	-	-	-	-	-	-	-	+	-	-	+	-

[a] The complementation pattern, as inferred by restoration of ethionine sensitivity, is reproduced from Table 13 together with results on repression of the *metB*, *metC*, and *metK* genes (Table 14). An encircled positive response (⊕) indicates complementing *metJ/metJ* merodiploids which have wild type levels of enzyme activities. A boxed response (⊞) indicates somewhat higher levels of enzyme activities. The mutants are arranged (left to right, and top to bottom) according to their linear order along the *metJ* gene.

Restrictive complementation in ethionine sensitive metJ/metJ merodiploids: Selective repression of the metA gene

The structural genes coding for the methionine biosynthetic enzymes in *E. coli* are scattered around the chromosome. This non-contiguous arrangement of genes is likely to entail individual operator sequences, located adjacent to each structural gene, which are recognized by a common *met* repressor protein. Since these genes have been shown to be repressed in a non-coordinate fashion, it would appear that the different operator sequences may have different affinities for the *met* repressor. Therefore it is conceivable then that an altered repressor, such as that produced by two complementing *metJ* mutants, may exhibit differential alteration in its recognition and binding affinity for the various operator sequences.

The complementation pattern of the *metJ* mutants, as inferred by restoration of ethionine sensitivity (Table 13), is reproduced in Table 18 together with results on repression of the *metA*, *metB*, *metC*, and *metK* specified enzymes (Table 19, Appendix II). These results show that several complementing *metJ/metJ* merodiploids continued to exhibit elevated enzyme levels even though they had regained ethionine sensitivity. The enzyme activities of some *metJ/metJ* merodiploids, including both the *amber* and non-*amber metJ* alleles, are compared to the wild type in Table 19. It can be seen that the *metB*, *metC*, and *metK* specified enzymes were still derepressed although the *metA* enzyme was repressed. Enzyme levels in other complementing merodiploids, where information is less complete, are summarized in Appendix II.

TABLE 18 - *metJ/metJ* merodiploids exhibiting restrictive complementation which leads to selective repression of the *metA* gene only[a]

	F14 episomal *metJ* alleles															
Chromosomal *metJ* alleles	*1275*	*1249*	*1284*	*1256*	*148*	*1257*	*1258*	*1264*	*1277*	*1279*	*1252*	*36*	*120*	*1271*	*1245*	*101*
1275	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	⊕
1284	-	-	-	-	⊕	+	+	-	⊕	+	-	-	+	-	+	⊕
*65*am	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1256	-	-	-	-	-	-	-	-	-	-	-	-	+	-	⊕	+
148	-	-	⊕	-	-	-	-	⊕	-	-	-	-	-	-	-	-
1257	-	-	+	-	-	-	-	-	-	⊕	-	-	-	-	-	-
1258	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1264	-	-	-	-	⊕	-	-	-	-	-	-	-	+	-	⊕	-
1277	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1279	-	-	+	-	-	+	-	-	-	-	-	+	-	-	⊕	-
1252	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*93*am	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
*1209*am	⊕	⊕	+	-	⊕	-	-	⊕	⊕	⊕	-	-	+	⊕	⊕	-
*89*am	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-
36	-	-	-	-	-	-	-	-	-	+	-	-	+	-	⊕	⊕
120	-	-	+	+	-	-	-	+	-	-	-	+	-	-	⊕	-
1271	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1245	-	-	+	⊕	-	-	-	⊕	-	⊕	-	⊕	⊕	-	-	⊕
101	-	⊕	⊕	+	-	-	-	-	-	-	-	⊕	-	-	⊕	-

---

[a] The complementation pattern, as inferred by restoration of ethionine sensitivity (Table 13) is presented together with results on repression of the *metA*, *metB*, *metC*, and *metK* genes (Table 19, Appendix II). Encircled positive responses (⊕) indicate combinations of *metJ* alleles which exhibit partial complementation, such that the *metB*, *metC*, and *metK* enzymes were found at constitutive levels, whereas the activity of the *metA* enzyme (homoserine-O-transsuccinylase) was found to be comparable to the wild type. The mutants are arranged (left to right, and top to bottom) according to their linear order along the *metJ* gene.

TABLE 19 - Enzyme activities of ethionine sensitive *metJ/metJ* merodiploids exhibiting restrictive complementation leading to selective repression of the *metA* gene only

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]			
		Homoserine-*O*-transsuccinylase (*metA*)	Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
metJ^{+}	*metJ*$^{+}$[b]	1.0	1.0	1.0	1.0
metJ36	*metJ1245*	1.5	4.9	7.1	2.8
metJ101	*metJ1249*	1.1	4.5	14.4	2.4
metJ101	*metJ36*	1.4	7.1	7.9	1.9
metJ^{+}	*metJ*$^{+}$[c]	1.0	1.0	1.0	1.0
metJ1264	*metJ1209*$_{am}$	1.1	6.3	6.2	2.0

[a] The activity of each enzyme is expressed relative to the specific activity of the wild type which is taken as 1.0.

[b] The recipient strains were *su*$^{+}$ and the actual specific activities of the *metJ*$^{+}$/*metJ*$^{+}$ wild type were: homoserine-*O*-transsuccinylase, 531.7; cystathionine-γ-synthetase, 0.283; β-cystathionase, 0.102; and ATP:Methionine *S*-adenosyltransferase, 11.39.

[c] The recipient strains were *su*^{o} and the actual specific activities of the *metJ*$^{+}$/*metJ*$^{+}$ wild type were: homoserine-*O*-transsuccinylase, 160.4; cystathionine-γ-synthetase, 0.175; β-cystathionase, 0.112; and ATP:Methionine *S*-adenosyltransferase, 10.76. The units of specific activities are described under "Materials and Methods."

The ethionine sensitivity of these *metJ/metJ* merodiploids must, therefore, be attributed to the selective repression of the crucial first step (i.e., the *metA* gene expression) by the partially functional repressor. The derepressed levels of enzymes catalyzing subsequent steps of the pathway suggest that the early intermediate, *O*-succinylhomoserine, was probably limiting the synthesis of methionine. In order to test this hypothesis these *metJ/metJ* merodiploids were tested for ethionine sensitivity in the presence of the various methionine biosynthetic intermediates (viz., homoserine, cystathionine, or homocysteine) and vitamin B12, or homocysteine plus vitamin B12. If the basis of observed ethionine sensitivity was the production of limiting quantities of *O*-succinylhomoserine due to repression of the *metA* gene, then the *metJ/metJ* merodiploids would be expected to be ethionine resistant in the presence of cystathionine, homocysteine, or homocysteine plus vitamin B12, but sensitive on homoserine or vitamin B12 alone. All of the *metJ/metJ* merodiploids (included in Table 19 and Appendix II) were found to be resistant to ethionine in the presence of cystathionine, homocysteine, and homocysteine plus vitamin B12; and ethionine sensitive in the presence of homoserine or vitamin B12. The wild type *metJ*$^+$/*metJ*$^+$ merodiploid was found to be ethionine sensitive in the presence of all of these supplements. These results provide additional support for the view that the availability of *O*-succinylhomoserine was limiting methionine biosynthesis in these *metJ/metJ* merodiploids, resulting in their ethionine sensitive phenotype. The results presented in Table 20 show that the overall production of methionine in four *metJ/metJ* merodiploids of this kind, as judged by measurements of endogenous

TABLE 20 - Methionine production in ethionine sensitive *metJ/metJ* merodiploids exhibiting restrictive complementation

Strain	Relative methionine production[a]	
	Intracellular pool size	Excretion into the medium
A. Chromosome *su*$^+$:		
F14 *metJ*$^+$/*metJ*$^+$	1.0[b]	1.0[c]
F14 *metJ36/metJ1245*	1.3	1.4
F14 *metJ101/metJ1249*	1.4	1.3
F14 *metJ101/metJ36*	1.2	1.3
B. Chromosome *su*^{o}:		
F14 *metJ*$^+$/*metJ*$^+$	1.0[d]	1.0[e]
F14 *metJ1264/metJ1209*$_{am}$	1.0	0.9

[a] The endogenous methionine pool sizes and methionine excretion are expressed relative to the wild type levels, which are taken as 1.0. The actual values for the wild type are given below.

[b] 0.071 nmoles methionine per mg wet weight of bacteria.

[c] 0.074 nmoles methionine excreted per ml (3-4 x 10^9 cells) of culture medium.

[d] 0.066 nmoles methionine per mg wet weight of bacteria.

[e] 0.067 nmoles methionine excreted per ml (3-4 x 10^9 cells) of culture medium.

free methionine pools and excretion of the amino acid, were comparable to the wild type. These results indicate that in these complementing *metJ/metJ* merodiploids, the *metA* gene is efficiently repressed in a manner similar to the wild type. Unfortunately, this hypothesis could not be tested directly on ethionine plates supplemented with *O*-succinylhomoserine since the latter compound, when supplied exogenously, is not utilized by *E. coli* in an efficient manner (Flavin, 1971).

Influence of the location of metJ alleles on the episome or chromosome on complementation

Among the various *metJ/metJ* merodiploids tested, two mutant combinations (viz., *metJ1257* with *metJ1279*, and *metJ1277* with *metJ1284*) were unique in the sense that restoration of repression was dependent upon the location of the specific *metJ* allele on the episome or the chromosome. Thus, in F14 *metJ1257/metJ1279* and F14 *metJ1284/metJ1277* merodiploids, the methionine biosynthetic enzymes appeared to be fully repressed like the wild type (Table 21). However, when the *metJ* alleles were switched around, i.e., in F14 *metJ1279/metJ1257* and F14 *metJ1277/metJ1284* merodiploids, complementation was restrictive. In these merodiploids, the *metB*, *metC*, and *metK* enzymes were synthesized in a constitutive manner, but the first enzyme (*metA*) was repressed. The data presented in Table 22 show that, in these merodiploids, the intracellular methionine pools and the excretion of methionine were similar to the wild type level. The F14 *metJ1279/metJ1257* and F14 *metJ1277/metJ1284* merodiploids were found to be resistant to ethionine in the presence of cystathionine, homocysteine,

TABLE 21 - Enzyme activities of complementing *metJ/metJ* merodiploids in which the location of the *metJ* allele on the episome or the chromosome seems to influence the extent and the pattern of repression

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]			
		Homoserine-*O*-transsuccinylase (*metA*)	Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
metJ$^+$	*metJ*$^+$	1.0	1.0	1.0	1.0
metJ1257	*metJ1279*	-	0.5	1.0	0.7
metJ1279	*metJ1257*	1.3	5.4	13.4	1.6
metJ1284	*metJ1277*	-	1.5	1.4	1.6
metJ1277	*metJ1284*	0.7	5.9	7.0	2.3

[a] The activity of each enzyme is expressed relative to the specific activity of the wild type which is taken as 1.0. The actual specific activities of the wild type were: homoserine-*O*-transsuccinylase, 531.7; cystathionine-γ-synthetase, 0.283; β-cystathionase, 0.102; and ATP:Methionine *S*-adenosyltransferase, 11.39. The units of specific activities are defined under "Materials and Methods."

TABLE 22 - Methionine production in *metJ/metJ* merodiploids

Strain	Relative methionine production[a]	
	Intracellular pool size	Excretion into the medium
F14 *metJ*$^+$/*metJ*$^+$	1.0[b]	1.0[c]
F14 *metJ1279/metJ1257*	1.0	1.1
F14 *metJ1277/metJ1284*	0.9	1.0

[a] The endogenous methionine pool sizes and methionine excretion are expressed relative to the wild type levels, which are taken as 1.0. The actual values for the wild type are given below.

[b] 0.071 nmoles methionine per mg wet weight of bacteria.

[c] 0.074 nmoles methionine excreted per ml (3-4 x 10^9 cells) of culture medium.

or homocysteine plus vitamin B12, and sensitive in the presence of homoserine or vitamin B12. This observation indicates that, in these merodiploids, the synthesis of methionine is indeed limited by the supply of *O*-succinylhomoserine. On the other hand, the alternate *metJ/metJ* allelic combinations (viz., F14 *metJ1257/metJ1279* and F14 *metJ1284/metJ1277*) were ethionine sensitive in the presence of all of these intermediates. Apparently, differences observed in the patterns of regulation in the two episomal-chromosomal combinations arise from gene dosage effects or more efficient expression in one of the two locations.

DISCUSSION

1. *Mapping of the metJ gene*

A series of mutants within the *metJ* gene of *E. coli* have been investigated in order to obtain information about the nature of the product specified by this gene. Previous evidence seems to indicate that this gene codes for the cytoplasmic repressor of methionine biosynthesis (Chater, 1970; Su and Greene, 1971; Ahmed, 1973).

Mutants defective in the *metJ* gene can be easily isolated by selection for ethionine resistance (Lawrence *et al.*, 1968; Ahmed and Duncan, 1968; Holloway *et al.*, 1970; Ahmed, 1973). The basis of this selection procedure seems to be as follows. Ethionine is a structural analog of methionine which competitively inhibits methionyl-tRNA synthetase in the formation of methionyl-tRNA and the misincorporation of ethionine into proteins, caused by the formation of small quantities of ethionyl-tRNA, results in cell death (Spizek and Janecek, 1969). The *metJ* mutants synthesize the methionine biosynthetic enzymes in a constitutive manner. Therefore, these mutants acquire high intracellular pools of methionine which greatly reduces or eliminates the misincorporation of ethionine.

Out of 90 ethionine resistant mutants of spontaneous origin selected from strain CSH4, 45 were classifed as *metJ* on the basis of cotransduction (92 - 100%) with the *metB* locus (Table 2). Seventeen of these *metJ* mutants were assayed for N^5,N^{10}-methylene-tetrahydrofolate reductase and β-cystathionase activities and were

found to exhibit greatly elevated levels of the two enzymes (Table 3). This confirms that the *metJ* gene, in its wild type state, regulates the expression of the *metF* and *metC* loci. Of the 45 *metJ* mutants tested, one (i.e., AA1209) was found to carry an *amber* mutation. This allele provides additional evidence that the *met* repressor, encoded by the *metJ* gene, is a protein (Minson and Smith, 1972; Morowicz, 1975).

Two different orientations of the *metJ* gene in *E. coli* with respect to adjacent loci have been published. Su and Greene (1971) reported the gene order *metB metJ metF*, and Ahmed (1973) suggested the gene sequence *metJ metB metF*, which is identical to the sequence found in *S. typhimurium*. Subsequent experiments based on the analysis of 4-point transduction crosses (Ahmed, unpublished), however, suggested that the sequence in *E. coli* was *metB metJ metF*. This latter sequence was confirmed by additional 4-point crosses, and the unambiguous sequence of genes in this region is concluded to be *glpK metB metJ* (*metL,M*) *metF argH* in the clockwise direction on the *E. coli* genetic map (Taylor and Trotter, 1972).

2. *Genetic fine structure analysis of the metJ gene*

Four-point transduction crosses were carried out to determine the linear order of 20 mutational sites within the *metJ* gene with respect to the flanking markers *glpK* and *argH* (Table 8, Appendix I; Figures 5 and 6). These *metJ* mutants include 4 *amber* mutants and 16 non-*amber* mutants (Figure 7). The relative position of the various mutations on the fine structure genetic map of the

metJ gene did not reveal any apparent correlation with the extent of observed derepression for various *met* biosynthetic enzymes, viz., cystathionine-γ-synthetase, β-cystathionase, and ATP:Methionine *S*-adenosyltransferase (Table 7).

The results obtained do not permit any definitive conclusions regarding the existence of any polycistronic transcription from the *metB metJ* (*metL,M*) *metF* gene cluster. The internal location of the *metJ* gene in this cluster predicts that some nonsense polar mutations, located at one end of the *metJ* gene, could cause methionine auxotrophy by polarity if this entire cluster was transcribed as one mRNA. Therefore, such polar *metJ* mutations would not be isolated by the selection procedure. However, the observation that the 4 *amber metJ* mutants studied were distributed at both ends of the genetic map (*metJ65*$_{\mathrm{am}}$ maps toward the left end, i.e., next to *metB*, whereas *metJ93*$_{\mathrm{am}}$, *metJ1209*$_{\mathrm{am}}$, and *metJ89*$_{\mathrm{am}}$ map toward the right end, i.e., next to *metF*) and that no polar effects on the *metB* and *metF* genes were discernible suggests that this entire region is not transcribed as one unit. This reasoning does not rule out the possibility that because of the existence of multiple isofunctional aspartokinases and homoserine dehydrogenases (Cohen, 1969), polar effects of nonsense *metJ* mutations could conceivably extend into *metL,M*, or *metL,M* mutations could exert polar effects on *metJ*. This would not cause methionine auxotrophy, nor would this be easily detected since the cell could compensate for the lost function by derepression of other aspartokinases and homoserine dehydrogenases.

3. *Dominance of metJ mutants*

The *met* repressor has been shown to be a cytoplasmic molecule which acts in a negative manner as demonstrated by the dominance of the wild type allele in most *metJ*$^+$/*metJ*$^-$ merodiploids (Chater, 1970; Su and Greene, 1971; Ahmed, 1973). However, two exceptional *metJ* mutants have been reported. Chater (1970) reported a mutant of *S. typhimurium*, and Morowicz (1975), from this laboratory, described an *amber* mutant of *E. coli*, both of which appeared to be dominant *metJ* mutations. In *metJ*$^+$/*metJ*$^-$ merodiploids, these mutants continued to exhibit ethionine resistance and constitutive enzyme synthesis. This was interpreted to mean that the *met* repressor may be an oligomeric protein.

The *metJ* mutants included in this work were tested for dominance by constructing *metJ*$^+$/*metJ*$^-$ merodiploids. Of the 20 mutants tested, 3 (viz., *metJ65*$_{\text{am}}$, *metJ1271*, and *metJ1275*) exhibited dominance over *metJ*$^+$, as indicated by ethionine resistance (Table 10). The two non-*amber* alleles, *metJ1271* and ***metJ1275*** exhibited dominance only when located on the episome. Thus, F14 *metJ1271*/*metJ*$^+$ and F14 *metJ1275*/*metJ*$^+$ merodiploids were ethionine resistant, derepressed for the *metA* (5 fold), *metB* (4-5 fold), *metC* (3-5 fold), and *metK* (2 fold) enzymes (Table 11), and produced 3-4 times the amount of free methionine as compared to the wild type (Table 12). However, when these alleles were located on the chromosome (i.e., in F14 *metJ*$^+$/*metJ1271* and F14 *metJ*$^+$/*metJ1275* merodiploids), they appeared recessive to the wild type, as indicated by ethionine sensitivity, repressed enzyme levels (Table 11), and normal methionine production in the merodiploids (Table 12). These results are consistent with

the interpretation that the active form of the *metJ* repressor is oligomeric. It appears that *metJ1271* and *metJ1275* exhibit *trans*-dominance, when located on the episome, because of the production of large numbers of copies of defective monomers which inactivate the normal ($metJ^+$) subunits by aggregation. The over-production of defective *metJ* monomers is most probably a consequence of gene dosage effects due to the presence of more than one copy of the episome per chromosome. In this respect, these 2 *metJ* alleles behave in a manner analogous to the episomal *trans*-dominant regulatory mutants affecting capsular polysaccharides described by Markovitz and Rosenbaum (1965).

The dominance of the *amber* $metJ65_{am}$ allele, as indicated by ethionine resistance and constitutive enzyme synthesis (Table 11) in F14 $metJ^+/metJ65_{am}$ merodiploids, confirm the earlier description of this mutant by Morowicz (1975). In addition, the results reported here show that F14 $metJ^+/metJ65_{am}$ merodiploids produce three times more methionine than the wild type (Table 12). The dominance of the $metJ65_{am}$ allele could be due to the association of the prematurely terminated polypeptide fragments or most probably the restart fragments with wild type subunits to produce nonfunctional hybrid repressors.

Previous work on the *lac* repressor has shown that the *trans*-dominant *lac* i^{-d} mutants map very near the amino-terminal end of the *lac i* gene (Müller-Hill *et al.*, 1968; Pfahl, 1972). This region of the *lac* repressor molecule has been shown to be involved in the recognition of the *lac* operator by the repressor

(Müller-Hill *et al.*, 1968; Adler *et al.*, 1972), and as a consequence, the *lac* i^{-d} mutants are unable to bind the operator and are constitutive. The *trans*-dominance of nonsense *lac* i^{-d} mutants was clearly shown to be due to the aggregation of restart fragments with the wild type monomers resulting in the formation of defective *lac* repressors (Platt *et al.*, 1972; Ganem *et al.*, 1973; Files *et al.*, 1974).

The locations of the dominant *metJ* mutations on the genetic map are not completely analogous to the map positions of the *lac* i^{-d} mutants. The alleles *metJ1275* and *metJ65*$_{am}$ map at the left end of the *metJ* genetic map (i.e., toward the *metB* locus), whereas, *metJ1271* maps at the right end of the genetic map (i.e., toward the *metF* locus), as shown in Figure 7. This distribution of the dominant *metJ* mutants at the opposite ends of the *metJ* gene suggests that both the amino- and carboxy-termini of the repressor are crucial to its function. In particular, these results indicate that the repressors produced in the various dominant mutants may be defective in (i) the recognition and binding of the corepressor, or (ii) recognition and binding to the operator sequences of the *met* structural genes. It is conceivable that the two functions reside in the opposite termini of the repressor polypeptide chain, and that the two groups of dominant mutants are defective in either one of the two functions. Purification of the normal and defective *met* repressors and *in vitro* experiments would clearly provide the final answer.

4. *Intra-cistronic complementation*

Intra-cistronic complementation between *metJ* mutants was studied by constructing *metJ/metJ* merodiploids in all possible pairwise combinations. Sixteen non-*amber metJ* mutants located on F14 episomes were tested against 20 recipient strains harboring the same 16 *metJ* alleles in addition to 4 *amber metJ* alleles. Complementation between *metJ* mutants was recognized by the restoration of ethionine sensitivity, and lack of complementation was indicated by continual ethionine resistance of the merodiploids. The complementation pattern, obtained by this criterion, was found to be complex (Table 13). The complementing mutants did not form distinct clusters on the genetic map and no evidence for polarity was found. These results appear to be typical of intra-cistronic complementation, suggesting that the *metJ* gene represents a single cistron which specifies one continuous polypeptide chain.

The occurrence of intra-cistronic complementation has been attributed to the association of mutationally defective protomers to reconstitute oligomers which regain partial function (Crick and Orgel, 1964). One of the first demonstrations of intra-cistronic complementation was the formation of active hybrid dimers of *E. coli* alkaline phosphatase both *in vivo* (Garen and Garen, 1963) and *in vitro* (Schlesinger and Levinthal, 1963). In this as well as other documented cases the normal protein was a multimer consisting of two or more identical subunits (Bourgeois *et al.*, 1965; Müller-Hill *et al.*, 1968; Ullman and Perrin, 1970; Yanofsky and Crawford, 1972; Zabin and Villarego, 1975; Gibbons *et al.*, 1975). In order to

estimate the functional capacities of *met* repressors produced in various complementing *metJ* mutants, the levels of the methionine biosynthetic enzymes in various *metJ/metJ* merodiploids were determined. As expected, several of the merodiploids exhibited wild type levels of the *metA*, *metB*, *metC*, and *metK* enzymes (Table 14), and methionine production (Table 15). The results clearly indicate the presence of functional repressors in *metJ/metJ* merodiploids, and it is concluded that two mutationally altered *metJ* protomers are capable of forming hybrid oligomeric repressors which can restore repression. These hybrid *met* repressors must have the ability to bind both the corepressor and the operator sequences of the *met* structural genes with reasonable efficiency. As expected, all of the mutants exhibiting intra-cistronic complementation were recessive to the wild type.

Surprisingly, several F14 $metJ/metJ_{am}$ merodiploids, each containing one of the 3 *amber* alleles (viz., $metJ89_{am}$, $metJ93_{am}$, or $metJ1209_{am}$), exhibited restoration of methionine repression. The activities of the *metB*, *metC*, and *metK* enzymes in these merodiploids were comparable to the wild type (Table 16). This indicates that the prematurely terminated *amber* fragments, or the restart fragments produced by these $metJ_{am}$ mutants were capable of complementing other (non-*amber*) defective protomers. These 3 *amber metJ* mutants, unlike the *trans*-dominant $metJ65_{am}$ mutant, were recessive to the wild type allele as indicated by the ethionine sensitivity and wild type levels of enzyme activities in the F14 $metJ^{+}/metJ_{am}$ merodiploids (Table 10 and 16), and they mapped as a cluster on the genetic map (Figure 7).

Taken together, the results of intra-cistronic complementation indicate that: (i) the *metJ* gene is a single cistron which specifies one polypeptide chain; (ii) these polypeptide chains are capable of aggregating to produce the functional *met* repressor; (iii) intra-cistronic complementation between *metJ* mutants probably occurs by the aggregation of two kinds of defective protomers to reconstitute the functional repressor; and (iv) complementation can also occur by the aggregation of an incomplete polypeptide chain produced by an *amber* mutant or a re-initiation fragment following premature termination, with a non-*amber* polypeptide to produce a functional aggregate capable of restoring repression.

5. *Examples of "restrictive" complementation*

The non-contiguous arrangement of *met* structural genes on the *E. coli* chromosome suggest that separate operator sequences, located adjacent to each structural gene, may be the receptor sites for the *met* repressor. Since these genes have been shown to be repressed in a non-coordinate fashion (Lawrence *et al.*, 1968; Holloway, 1970; Ahmed, 1973), it would appear that the repressor may have different recognition and binding affinities for the individual operators. Therefore, it is conceivable that an altered repressor, such as that produced by two complementing *metJ* mutants, may exhibit differential restoration of its recognition and binding affinities for the various operators. This appears to be the case for several ethionine sensitive *metJ/metJ* merodiploids (Table 18), and this phenomenon is referred to as "restrictive" intra-cistronic

complementation. Although phenotypically wild type (i.e., ethionine sensitive), these merodiploids exhibited constitutive levels of the *metB*, *metC*, and *metK* enzymes (Table 19; Appendix II). However, the *metA* enzyme (homoserine-*O*-transsuccinylase) and overall methionine production in these merodiploids were comparable to the wild type (Tables 19 and 20). The ethionine sensitivity of these merodiploids was, therefore, caused by the *selective* repression of the *metA* gene, which resulted in the synthesis of limiting amounts of *O*-succinylhomoserine. Evidence in support of this interpretation was obtained by the demonstration that, unlike the wild type, these merodiploids turned ethionine resistant when supplied with exogenous cystathionine or homocysteine, whereas supplying homoserine (the precursor of *O*-succinylhomoserine) failed to confer ethionine resistance. These experiments indicate that the *met* repressors produced in *metJ/metJ* merodiploids which exhibit restrictive complementation have regained affinities for the corepressor and only the *metA* operator, but not for the operators of the remaining *met* structural genes. This implies that the operator sequence of the *metA* gene might be different from the operator sequences of the other *met* structural genes, or that the *metA* gene is subject to an additional regulatory mechanism which involves the *met* repressor and which is not operational on other *met* genes.

Restrictive intra-cistronic complementation was also exhibited by several combinations of various non-*amber metJ* alleles with the *amber* $metJ1209_{\mathrm{am}}$ mutant (Table 18). Interestingly, the two dominant non-*amber* alleles (viz., *metJ1271* and *metJ1275*) also exhibited restrictive

complementation with *metJ1209*$_{am}$. This indicates that the *amber* fragment produced by *metJ1209*$_{am}$ can also interact with protomers which apparently have gross deformation as manifested by their dominance over the wild type.

Among the various complementing *metJ* mutant combinations tested, 2 mutant combinations (viz., *metJ1257/metJ1279*, and *metJ1277/metJ1284*) were unique in that restoration of repression was complete or partial depending upon the locations of the *metJ* alleles on the episome or the chromosome. In F14 *metJ1257/metJ1279* and F14 *metJ1284/metJ1277* merodiploids, repression was apparently normal because the methionine biosynthetic enzymes were fully repressed (Table 21), and the merodiploids were ethionine sensitive even in the presence of all of the methionine biosynthetic intermediates. However, when the *metJ* alleles were switched around (viz., in F14 *metJ1279/metJ1257* and F14 *metJ1277/metJ1284* merodiploids), complementation was restrictive. This was apparent because the *metA* enzyme was repressed and the *metB*, *metC*, and *metK* enzymes were constitutive (Table 21), the overall production of methionine was similar to the wild type (Table 22), and the merodiploids were ethionine resistant in the presence of cystathionine or homocysteine. The different patterns of partial complementation in the two episomal/chromosomal combinations are probably due to gene dosage effects discussed earlier.

6. *General conclusions*

To summarize, the present studies lead to the following conclusions: (i) the correct sequence of the loci in the vicinity

of the *metJ* gene (min 78) on the *E. coli* K12 chromosome is *glpK metB metJ* (*metL,M*) *metF argH*; (ii) various independent sites of mutation affecting the *metJ* gene can be arranged in an unambiguous sequence by 4-point crosses; (iii) the *metJ* gene represents one cistron which codes for the cytoplasmic *met* repressor; (iv) the protein specified by the *metJ* gene shows subunit interaction indicating that the active repressor is an oligomer; (v) differentially defective repressor protomers can complement by aggregation to reconstitute functional hybrid repressors; (vi) this aggregation does not always require complete polypeptide chains, and can occur between a complete protomer and a fragment produced by premature termination at *amber* mutations, or reinitiation fragments following premature termination; and (vii) aggregation between differentially defective protomers can often lead to only partial restoration of repression so that only the first gene of the *met* pathway is repressed while others remain constitutive. This suggests that either the operator site of the *metA* gene is different from other *met* genes, or that the first gene is subject to another regulatory mechanism which involves the participation of the *met* repressor.

BIBLIOGRAPHY

ADLER, K., K. BEYREUTHER, E. FANNING, N. GEISLER, B. GRONENBORN, A. KLEMM, B. MULLER-HILL, M. PFAHL, and A. SCHMITZ, 1972. How *lac* repressor binds to DNA. Nature *237*: 322-327.

AHMED, A., 1973. Mechanism of repression of methionine biosynthesis in *Escherichia coli*. I. The role of methionine, *S*-adenosylmethionine, and methionyl-transfer ribonucleic acid in repression. Molec. Gen. Genet. *123*: 299-324.

AHMED, A. and M. DUNCAN, 1968. Regulation of methionine biosynthesis in *Escherichia coli*. In: *Proceedings of the International Congress of Genetics XII,* Vol. I, p. 23. Tokyo.

ARTZ, S.W. and J.R. BROACH, 1975. Histidine regulation in *Salmonella typhimurium*: An activator-attenuator model of gene regulation. Proc. Nat. Acad. Sci. U.S.A. *72*: 3453-3457.

BECKWITH, J.R., 1964. A deletion analysis of the *lac* operator region in *Escherichia coli*. J. Mol. Biol. *8*: 427-430.

BECKWITH, J.R. and P. ROSSOW, 1974. Analysis of genetic regulatory mechanisms. Ann. Rev. Genet. *8*: 1-13.

BERTRAND, K., L. KORN, F. LEE, T. PLATT, C.L. SQUIRES, C. SQUIRES, and C. YANOFSKY, 1975. New features of the regulation of the tryptophan operon. Science *189*: 22-26.

BOURGEOIS, S., M. COHN, and L.E. ORGEL, 1965. Suppression of and complementation among mutants of the regulatory gene of the lactose operon of *Escherichia coli*. J. Mol. Biol. *14*: 300-302.

BRENNER, M. and B.N. AMES, 1971. The histidine operon and its regulation. In: *Metabolic Pathways*, Vol. 5, p. 349-387. Edited by H.J. Vogel. Academic Press Inc., New York.

BRENNER, M. and B.N. AMES, 1972. Histidine regulation in *Salmonella typhimurium*. IX. Histidine transfer ribonucleic acid in the regulatory mutants. J. Biol. Chem. *247*: 1080-1088.

CANTONI, G.L., 1965. *S*-adenosylmethionine revisited. In: *Transmethylation and methionine biosynthesis*, p. 21-32. Edited by S.K. Shapiro and F. Schlenk. Chicago University Press, Chicago.

CHATER, K.F., 1970. Dominance of the wild type alleles of methionine regulatory genes in *Salmonella typhimurium*. J. Gen. Microbiol. *63*: 95-109.

CHATER, K.F. and R.J. ROWBURY, 1970. A genetical study of the feed-back sensitive enzyme of methionine synthesis in *Salmonella typhimurium*. J. Gen. Microbiol. *63*: 111-120.

COHEN, G., 1969. The aspartokinases and homoserine dehydrogenases of *Escherichia coli*. In: *Current Topics in Cellular Regulation,* Vol. 1, p. 183-231. Edited by B.L. Horecker and E.R. Stadtman. Academic Press Inc., New York.

COHEN, G. and F.JACOB, 1959. Sur la répression de la synthèse des enzymes intervenant dans la formation du tryptophane chez *Escherichia coli*. C.R. Acad. Sci. (Paris) *248*: 3490-3492.

CRICK, F.H.C. and L.E. ORGEL, 1964. The theory of inter-allelic complementation. J. Mol. Biol. *8*: 161-165.

DE LORENZO, F., D. STRAUS, and B.N. AMES, 1972. Histidine regulation in *Salmonella typhimurium* X. Kinetic studies of mutant histidyl transfer ribonucleic acid synthetases. J. Biol. Chem. *247*: 2302-2307.

DICKERMAN, H. and H. WEISSBACH, 1964. Altered folate metabolism in a vitamin B12-methionine auxotroph. Biochem. Biophys. Res. Commun. *16*: 593-599.

EMMER, M., B. DE CROMBRUGGHE, I. PASTAN, and R. PERLMAN, 1970. The cyclic AMP receptor protein of *E. coli*: Its role in the synthesis of inducible enzymes. Proc. Nat. Acad. Sci. U.S.A. *66*: 480-487.

FILES, J.G., K. WEBER, and J.H. MILLER, 1974. Translational reinitiation: Reinitiation of *lac* repressor fragments at three internal sites early in the *lac i* gene of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. *71*: 667-670.

FLAVIN, M., 1962. Microbial transsulfuration: the mechanism of an enzymatic disulfide elimination reaction. J. Biol. Chem. *237*: 768-777.

FLAVIN, M., 1971. Miscellaneous procedures involved in transsulfuration. In: *Methods in Enzymology*, Vol. 17(B), p. 450-453. Edited by H. Tabor and C.W. Tabor. Academic Press Inc., New York.

FLAVIN, M., 1975. Methionine biosynthesis. In: *Metabolic Pathways*, Vol. 7, p. 457-503. Academic Press Inc., New York.

GANEM, D., J.H. MILLER, J.F. FILES, T. PLATT, and K. WEBER, 1973. Reinitiation of a *lac* repressor fragment at a codon other than AUG. Proc. Nat. Acad. Sci. U.S.A. *70*: 3165-3169.

GAREN, A. and S. GAREN, 1963. Complementation *in vivo* between structural mutants of alkaline phosphatase from *E. coli*. J. Mol. Biol. *7*: 13-22.

GIBBONS, I., J.E. FLATGAARD, and H.K. SCHACHMAN, 1975. Quaternary constraint in hybrid of aspartate transcarbamylase containing wild-type and mutant catalytic subunits. Proc. Nat. Acad. Sci. U.S.A. *72*: 4298-4302.

GILBERT, W. and B. MULLER-HILL, 1966. Isolation of the *lac* repressor. Proc. Nat. Acad. Sci. U.S.A. *56*: 1891-1898.

GOTS, J.S. and C.E. BENSON, 1974. Biochemical genetics of bacteria. Ann. Rev. Genet. *8*: 79-101.

HOBSON, A.C., 1974. The regulation of methionine and *S*-adenosylmethionine biosynthesis and utilization in mutants of *Salmonella typhimurium* with defects in *S*-adenosylmethionine synthetase. Molec. Gen. Genet. *131*: 263-273.

HOBSON, A.C. and D.A. SMITH, 1973. *S*-Adenosylmethionine synthetase in methionine regulatory mutants of *Salmonella typhimurium*. Molec. Gen. Genet. *126*: 7-18.

HOLCOMB, E.R. and S.K. SHAPIRO, 1975. Assay and regulation of *S*-adenosylmethionine synthetase in *Saccharomyces cerevisiae* and *Candida utilis*. J. Bacteriol. *121*: 267-271.

HOLLOWAY, C.T., R.C. GREENE, and C.H. SU, 1970. Regulation of *S*-adenosylmethionine synthetase in *Escherichia coli*. J. Bacteriol. *104*: 734-747.

JACOB, F. and J. MONOD, 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. *3*: 318-356.

KAPLAN, M.M. and M. FLAVIN, 1966. Cystathionine-γ-synthetase of *Salmonella*. J. Biol. Chem. *241*: 4463-4471.

LAWRENCE, D.A., D.A. SMITH, and R.J. ROWBURY, 1968. Regulation of methionine synthesis in *Salmonella typhimurium* mutants resistant to inhibition by analogues of methionine. Genetics *58*: 473-496.

LAYNE, E., 1957. Spectrophotometric and turbidimetric methods for measuring proteins. In: *Methods in Enzymology*, Vol. 3, p. 447-454. Edited by S.P. Colowick and N.O. Kaplan. Academic Press Inc., New York.

LEE, L.W., J.M. RAVEL, and W. SHIVE, 1966. Multimetabolite control of a biosynthetic pathway by sequential metabolites. J. Biol. Chem. *241*: 5479-5480.

LENNOX, E.S. and C. YANOFSKY, 1959. Transduction and recombination study of linkage relationships among the genes controlling tryptophan synthesis in *Escherichia coli*. Virology *8*: 425-447.

MAAS, W.K., 1972. Mapping of genes involved in the synthesis of spermidine in *Escherichia coli*. Molec. Gen. Genet. *119*: 1-9.

MARKOVITZ, A. and N. ROSENBAUM, 1965. A regulator gene that is dominant on an episome and recessive on a chromosome. Proc. Nat. Acad. Sci. U.S.A. *54*: 1084-1091.

MILLER, J.H., K. IPPEN, J.G. SCAIFE, and J.R. BECKWITH, 1968. The promotor-operator region of the *lac* operon of *Escherichia coli*. J. Mol. Biol. *38*: 413-420.

MINSON, A.C. and D.A. SMITH, 1972. Methionine regulatory defects in *Salmonella typhimurium* arising from amber-suppressible mutants. J. Gen. Microbiol. *70*: 471-476.

MOROWICZ, H.L., 1975. A study of nonsense regulatory mutations affecting methionine biosynthesis in *Escherichia coli*. M.Sc. Thesis. University of Alberta.

MORSE, D.E. and C. YANOFSKY, 1969. Amber mutants of the *trpR* regulatory gene. J. Mol. Biol. *44*: 185-193.

MULLER-HILL, B., L. CRAPO, and W. GILBERT, 1968. Mutants that make more *lac* repressor. Proc. Nat. Acad. Sci. U.S.A. *59*: 1259-1264.

NAGAI, S. and D. KERR, 1971. Homoserine transacetylase (*Neurospora*). In: *Methods in Enzymology*, Vol. 17(B), p. 442-445. Edited by H. Tabor and C.W. Tabor. Academic Press Inc., New York.

PFAHL, M., 1972. Genetic map of the lactose repressor gene (*i*) of *Escherichia coli*. Genetics *72*: 393-410.

PLATT, T., K. WEBER, D. GANEM, and J.H. MILLER, 1972. Translational restarts: AUG reinitiation of a *lac* repressor fragment. Proc. Nat. Acad. Sci. U.S.A. *69*: 897-901.

REZNIKOFF, W.S., 1972. The operon revisited. Ann. Rev. Genet. *6*: 133-156.

ROSE, J.K., C.L. SQUIRES, C. YANOFSKY, H.L. YANG, and G. ZUBAY, 1973. Regulation of *in vitro* transcription of the tryptophan operon by purified RNA polymerase in the presence of partially purified repressor and tryptophan. Nature New Biol. *245*: 133-137.

ROTH, J.R., 1970. Genetic techniques in studies of bacterial metabolism. In: *Methods in Enzymology*, Vol. 17(A), p. 3-35. Edited by H. Tabor and C.W. Tabor. Academic Press Inc., New York.

SAVIN, M.A., M. FLAVIN, and C. SLAUGHTER, 1972. Regulation of homocysteine biosynthesis in *Salmonella typhimurium*. J. Bacteriol. *111*: 547-556.

SCHLESINGER, M.J. and C. LEVINTHAL, 1963. Hybrid protein formation of *E. coli* alkaline phosphatase leading to *in vitro* complementation. J. Mol. Biol. *7*: 1-12.

SCHLESINGER, S., 1967. Inhibition of growth of *Escherichia coli* and of homoserine-*O*-transsuccinylase by α-methylmethionine. J. Bacteriol. *94*: 327-332.

SCHWARTZ, D.S. and J.R. BECKWITH, 1970. Mutants missing a factor necessary for the expression of catabolite-sensitive operons in *E. coli*. In: *The Lactose Operon*, p. 417-422. Edited by J.R. Beckwith and D. Zipser. Cold Spring Harbor Laboratory, New York.

SHIMIZU, Y., N. SHIMIZU, and M. HAYASHI, 1973. *In vitro* repression of transcription of the tryptophan operon by *trp* repressor. Proc. Nat. Acad. Sci. U.S.A. *70*: 1990-1994.

SINGER, C., G. SMITH, R. CORTESE, and B.N. AMES, 1972. Mutant $tRNA^{His}$ ineffective in repression and lacking two pseudouridine modification. Nature New Biol. *238*: 72-74.

SMITH, D.A., 1971. *S*-Amino acid metabolism and its regulation in *Escherichia coli* and *Salmonella typhimurium*. Advanc. Genet. *16*: 141-165.

SMITH, D.A., and J.D. CHILDS, 1966. Methionine genes and enzymes of *Salmonella typhimurium*. Heredity *21*: 265-286.

SPIZEK, J. and J. JANECEK, 1969. The effect of ethionine on the synthesis of β-galactosidase: formation of an immunologically cross-reacting protein. Biochem. Biophys. Res. Commun. *34*: 17-21.

SQUIRES, C.L., F.D. LEE, and C. YANOFSKY, 1975. Interaction of the *trp* repressor and RNA polymerase with the *trp* operon. J. Mol. Biol. *92*: 93-111.

STEPHENS, J.C., S.W. ARTZ, and B.N. AMES, 1975. Guanosine 5'-diphosphate 3'-diphosphate (ppGpp): Positive effector for histidine operon transcription and general signal for amino-acid deficiency. Proc. Nat. Acad. Sci. U.S.A. *72*: 4389-4393.

SU, C.H. and R.C. GREENE, 1971. Regulation of methionine biosynthesis in *Escherichia coli*: mapping of the *metJ* locus and properties of a *metJ*$^+$/*metJ*$^-$ diploid. Proc. Nat. Acad. Sci. U.S.A. *68*: 367-371.

TABOR, H. and C.W. TABOR, 1971. Enzymatic synthesis of radioactive *S*-adenosyl L-methionine. In: *Methods in Enzymology*, Vol. 17(B), p. 393-397. Edited by H. Tabor and C.W. Tabor. Academic Press Inc., New York.

TABOR, H., C.W. TABOR, and S.M. ROSENTHAL, 1961. The biochemistry of the polyamines: spermidine and spermine. Ann. Rev. Biochem. *30*: 579-604.

TAYLOR, A.L. and C.D. TROTTER, 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. *36*: 504-524.

THEZE, J., D. MARGARITA, G.N. COHEN, F. BORNE, and J.C. PATTE, 1974. Mapping of the structural genes of the three aspartokinases and of the two homoserine dehydrogenases of *Escherichia coli* K-12. J. Bacteriol. *117*: 133-143.

ULLMAN, A. and D. PERRIN, 1970. Complementation in β-galactosidase. In: *The Lactose Operon*, p. 143-172. Edited by J.R. Beckwith and D. Zipser. Cold Spring Harbor Laboratory, New York.

UMBARGER, H.E., 1971. Metabolic analogs as genetic and biochemical probes. Advanc. Genet. *16*: 119-136.

WHITEHOUSE, J.M. and D.A. SMITH, 1973. Methionine and vitamin B12 repression and precursor induction in the regulation of homocysteine methylation in *Salmonella typhimurium*. Molec. Gen. Genet. *120*: 341-353.

YANOFSKY, C. and I.P. CRAWFORD, 1972. Tryptophan synthetase. In: *The Enzymes*, Vol. 7, p. 1-31. Edited by P.D. Boyer. Academic Press Inc., New York.

ZABIN, I. and M.R. VILLAREJO, 1975. Protein complementation. Ann. Rev. Biochem. *44*: 295-313.

ZUBAY, G., D.E. MORSE, W. SCHRENK, and J. MILLER, 1972. Detection and isolation of the repressor protein for the tryptophan operon of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. *69*: 1100-1103.

ZUBAY, G., D.S. SCHWARTZ, and J.R. BECKWITH, 1970. Mechanism of activation of catabolite-sensitive genes: A positive control system. Proc. Nat. Acad. Sci. U.S.A. *66*: 104-110.

APPENDICES

APPENDIX I - Ordering of *metJ* alleles by four-point transduction crosses

Donor	Recipient	Selected marker	Number of trans-ductants tested	Number of metJ$^+$ recombi-nants found	Distribution of out-side markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ1275	*metJ1249*	argH$^+$	200	6		6	0	*glpK metJ1275 metJ1249 argH*
metJ1249	*metJ1275*	argH$^+$	248	3		0	3	" " " "
metJ1249	*metJ1275*	glpK$^+$	248	5	5		0	" " " "
metJ1275	*metJ148*	argH$^+$	200	3		3	0	*glpK metJ1275 metJ148 argH*
metJ148	*metJ1275*	glpK$^+$	248	4	3		1	" " " "
metJ1275	*metJ1258*	argH$^+$	248	4		3	1	*glpK metJ1275 metJ1258 argH*
metJ1258	*metJ1275*	argH$^+$	248	1		0	1	" " " "
metJ1275	*metJ1209*$_{am}$	argH$^+$	250	6		6	0	*glpK metJ1275 metJ1209*$_{am}$ *argH*
metJ1275	*metJ36*	argH$^+$	248	7		5	2	*glpK metJ1275 metJ36 argH*
metJ36	*metJ1275*	argH$^+$	248	3		0	3	" " " "
metJ1275	*metJ120*	argH$^+$	248	9		9	0	*glpK metJ1275 metJ120 argH*
metJ120	*metJ1275*	glpK$^+$	248	5	5		0	" " " "
metJ1249	*metJ1284*	argH$^+$	248	3		3	0	*glpK metJ1249 metJ1284 argH*
metJ1284	*metJ1249*	argH$^+$	200	2		0	2	" " " "
metJ1249	*metJ65*$_{am}$	argH$^+$	248	2		2	0	*glpK metJ1249 metJ65*$_{am}$ *argH*
metJ1249	*metJ1256*	argH$^+$	300	11		9	2	*glpK metJ1249 metJ1256 argH*
metJ1249	*metJ1256*	glpK$^+$	248	7	2		5	" " " "

APPENDIX I - continued

or	Recipient	Selected marker	Number of trans-ductants tested	Number of metJ^{+} recombi-nants found	Distribution of out-side markers in metJ^{+} recombinants			Order inferred
					glpK^{+} argH^{-}	glpK^{-} argH^{+}	glpK^{+} argH^{+}	
metJ1256	*metJ1249*	argH^{+}	200	2		0	2	*glpK metJ1249 metJ1256 argH*
metJ1256	*metJ1249*	glpK^{+}	248	3	3		0	" " " "
metJ1249	*metJ148*	argH^{+}	248	4		3	1	*glpK metJ1249 metJ148 argH*
metJ148	*metJ1249*	argH^{+}	248	3		0	3	" " " "
metJ1249	*metJ1258*	argH^{+}	250	3		2	1	*glpK metJ1249 metJ1258 argH*
metJ1258	*metJ1249*	argH^{+}	250	2		0	2	" " " "
metJ1249	*metJ93*$_{\text{am}}$	argH^{+}	200	3		3	0	*glpK metJ1249 metJ93*$_{\text{am}}$ *argH*
metJ1249	*metJ1209*$_{\text{am}}$	argH^{+}	200	2		2	0	*glpK metJ1249 metJ1209*$_{\text{am}}$ *argH*
metJ1249	*metJ89*$_{\text{am}}$	argH^{+}	200	4		4	0	*glpK metJ1249 metJ89*$_{\text{am}}$ *argH*
metJ1249	*metJ36*	argH^{+}	248	9		7	2	*glpK metJ1249 metJ36 argH*
metJ36	*metJ1249*	argH^{+}	248	8		3	5	" " " "
metJ36	*metJ1249*	glpK^{+}	248	5	5		0	" " " "
metJ1249	*metJ120*	argH^{+}	248	15		10	5	*glpK metJ1249 metJ120 argH*
metJ120	*metJ1249*	argH^{+}	248	11		2	9	" " " "
metJ1284	*metJ65*$_{\text{am}}$	argH^{+}	248	4		3	1	*glpK metJ1284 metJ65*$_{\text{am}}$ *argH*
metJ1256	*metJ1284*	argH^{+}	200	2		0	2	*glpK metJ1284 metJ1256 argH*
metJ1284	*metJ148*	argH^{+}	226	4		4	0	*glpK metJ1284 metJ148 argH*
metJ1284	*metJ36*	argH^{+}	248	8		7	1	*glpK metJ1284 metJ36 argH*

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of transductants tested	Number of metJ$^+$ recombinants found	Distribution of outside markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ1284	*metJ120*	argH$^+$	248	9		7	2	*glpK metJ1284 metJ120 argH*
metJ1256	*metJ65*$_{\text{am}}$	argH$^+$	248	5		1	4	*glpK metJ65*$_{\text{am}}$ *metJ1256 argH*
metJ148	*metJ65*$_{\text{am}}$	argH$^+$	248	3		0	3	*glpK metJ65*$_{\text{am}}$ *metJ148 argH*
metJ1277	*metJ65*$_{\text{am}}$	argH$^+$	248	4		1	3	*glpK metJ65*$_{\text{am}}$ *metJ1277 argH*
metJ65$_{\text{am}}$	*metJ93*$_{\text{am}}$	argH$^+$	213	4		3	1	*glpK metJ65*$_{\text{am}}$ *metJ93*$_{\text{am}}$ *argH*
metJ93$_{\text{am}}$	*metJ65*$_{\text{am}}$	argH$^+$	248	4		1	3	" " " "
metJ65$_{\text{am}}$	*metJ1209*$_{\text{am}}$	argH$^+$	248	3		2	1	*glpK metJ65*$_{\text{am}}$ *metJ1209*$_{\text{am}}$ *argH*
metJ1209$_{\text{am}}$	*metJ65*$_{\text{am}}$	argH$^+$	248	4		1	3	" " " "
metJ65$_{\text{am}}$	*metJ89*$_{\text{am}}$	argH$^+$	248	2		2	0	*glpK metJ65*$_{\text{am}}$ *metJ89*$_{\text{am}}$ *argH*
metJ89$_{\text{am}}$	*metJ65*$_{\text{am}}$	argH$^+$	248	3		1	2	" " " "
metJ1256	*metJ148*	argH$^+$	248	15		10	5	*glpK metJ1256 metJ148 argH*
metJ148	*metJ1256*	argH$^+$	248	6		2	4	" " " "
metJ1256	*metJ1279*	argH$^+$	200	3		3	0	*glpK metJ1256 metJ1279 argH*
metJ1256	*metJ1252*	argH$^+$	200	3		3	0	*glpK metJ1256 metJ1252 argH*
metJ1252	*metJ1256*	argH$^+$	248	4		1	3	" " " "
metJ1256	*metJ93*$_{\text{am}}$	argH$^+$	200	3		3	0	*glpK metJ1256 metJ93*$_{\text{am}}$ *argH*

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of transductants tested	Number of metJ$^+$ recombinants found	Distribution of outside markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ1256	*metJ36*	argH$^+$	248	2		2	0	*glpK metJ1256 metJ36 argH*
metJ36	*metJ1256*	argH$^+$	248	3		0	3	" " " "
metJ1256	*metJ120*	argH$^+$	248	1		1	0	*glpK metJ1256 metJ120 argH*
metJ120	*metJ1256*	argH$^+$	248	4		1	3	" " " "
metJ1256	*metJ101*	argH$^+$	248	12		9	3	*glpK metJ1256 metJ101 argH*
metJ101	*metJ1256*	argH$^+$	248	9		1	8	" " " "
metJ148	*metJ1257*	argH$^+$	248	4		4	0	*glpK metJ148 metJ1257 argH*
metJ1257	*metJ148*	argH$^+$	248	8		3	5	" " " "
metJ148	*metJ1258*	argH$^+$	248	5		4	1	*glpK metJ148 metJ1258 argH*
metJ1258	*metJ148*	argH$^+$	248	7		2	5	" " " "
metJ148	*metJ1264*	argH$^+$	200	5		4	1	*glpK metJ148 metJ1264 argH*
metJ1264	*metJ148*	argH$^+$	248	7		1	6	" " " "
metJ148	*metJ1277*	argH$^+$	200	4		3	1	*glpK metJ148 metJ1277 argH*
metJ1277	*metJ148*	argH$^+$	248	4		0	4	" " " "
metJ1279	*metJ148*	argH$^+$	224	4		0	4	*glpK metJ148 metJ1279 argH*
metJ148	*metJ1209*$_{am}$	argH$^+$	248	4		4	0	*glpK metJ148 metJ1209*$_{am}$ *argH*
metJ148	*metJ36*	argH$^+$	248	5		5	0	*glpK metJ148 metJ36 argH*
metJ148	*metJ36*	glpK$^+$	224	2	0		2	" " " "

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of transductants tested	Number of metJ$^+$ recombinants found	Distribution of outside markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ36	*metJ148*	argH$^+$	248	3		1	2	*glpK metJ148 metJ36 argH*
metJ36	*metJ148*	glpK$^+$	244	2	2		0	" " " "
metJ148	*metJ120*	argH$^+$	248	13		10	3	*glpK metJ148 metJ120 argH*
metJ148	*metJ120*	glpK$^+$	216	3	0		3	" " " "
metJ120	*metJ148*	argH$^+$	248	2		0	2	" " " "
metJ120	*metJ148*	glpK$^+$	242	11	10		1	" " " "
metJ148	*metJ1271*	argH$^+$	200	2		2	0	*glpK metJ148 metJ1271 argH*
metJ1271	*metJ148*	argH$^+$	248	4		0	4	" " " "
metJ148	*metJ101*	argH$^+$	248	12		10	2	*glpK metJ148 metJ101 argH*
metJ148	*metJ101*	glpK$^+$	208	2	0		2	" " " "
metJ101	*metJ148*	argH$^+$	280	10		1	9	" " " "
metJ101	*metJ148*	glpK$^+$	286	8	8		0	" " " "
metJ1257	*metJ1258*	argH$^+$	250	5		4	1	*glpK metJ1257 metJ1258 argH*
metJ1258	*metJ1257*	argH$^+$	248	5		0	5	" " " "
metJ1257	*metJ93*$_{am}$	argH$^+$	248	4		4	0	*glpK metJ1257 metJ93*$_{am}$ *argH*
metJ1257	*metJ36*	argH$^+$	248	1		1	0	*glpK metJ1257 metJ36 argH*
metJ36	*metJ1257*	argH$^+$	248	3		0	3	" " " "

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of transductants tested	Number of metJ$^+$ recombinants found	Distribution of outside markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ1257	*metJ120*	argH$^+$	226	1		1	0	*glpK metJ1257 metJ120 argH*
metJ120	*metJ1257*	argH$^+$	248	2		0	2	" " " "
metJ1258	*metJ1264*	argH$^+$	200	1		1	0	*glpK metJ1258 metJ1264 argH*
metJ1264	*metJ1258*	glpK$^+$	248	2	2		0	" " " "
metJ1258	*metJ1277*	argH$^+$	248	2		2	0	*glpK metJ1258 metJ1277 argH*
metJ1258	*metJ1252*	argH$^+$	200	4		4	0	*glpK metJ1258 metJ1252 argH*
metJ1252	*metJ1258*	argH$^+$	300	7		2	5	" " " "
metJ1258	*metJ93*$_{am}$	argH$^+$	200	2		2	0	*glpK metJ1258 metJ93*$_{am}$ *argH*
metJ1258	*metJ1209*$_{am}$	argH$^+$	250	3		3	0	*glpK metJ1258 metJ1209*$_{am}$ *argH*
metJ1258	*metJ36*	argH$^+$	248	4		4	0	*glpK metJ1258 metJ36 argH*
metJ36	*metJ1258*	argH$^+$	248	3		1	2	" " " "
metJ1258	*metJ120*	argH$^+$	248	7		6	1	*glpK metJ1258 metJ120 argH*
metJ120	*metJ1258*	argH$^+$	248	2		0	2	" " " "
metJ1258	*metJ1271*	argH$^+$	200	2		2	0	*glpK metJ1258 metJ1271 argH*
metJ1264	*metJ1277*	argH$^+$	248	3		3	0	*glpK metJ1264 metJ1277 argH*
metJ1277	*metJ1264*	argH$^+$	248	1		0	1	" " " "

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of trans-ductants tested	Number of metJ$^+$ recombi-nants found	Distribution of out-side markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ1264	*metJ93*$_{am}$	argH$^+$	248	4		3	1	*glpK metJ1264 metJ93*$_{am}$ *argH*
metJ1264	*metJ1209*$_{am}$	argH$^+$	248	2		2	0	*glpK metJ1264 metJ1209*$_{am}$ *argH*
metJ1264	*metJ36*	argH$^+$	248	9		7	2	*glpK metJ1264 metJ36 argH*
metJ36	*metJ1264*	argH$^+$	260	6		2	4	" " " "
metJ1264	*metJ120*	argH$^+$	248	3		3	0	*glpK metJ1264 metJ120 argH*
metJ120	*metJ1264*	argH$^+$	200	3		0	3	" " " "
metJ1277	*metJ1279*	argH$^+$	200	2		2	0	*glpK metJ1277 metJ1279 argH*
metJ1277	*metJ1252*	argH$^+$	248	3		3	0	*glpK metJ1277 metJ1252 argH*
metJ1277	*metJ93*$_{am}$	argH$^+$	248	3		3	0	*glpK metJ1277 metJ93*$_{am}$ *argH*
metJ1277	*metJ1209*$_{am}$	argH$^+$	248	4		4	0	*glpK metJ1277 metJ1209*$_{am}$ *argH*
metJ1277	*metJ36*	argH$^+$	248	6		5	1	*glpK metJ1277 metJ36 argH*
metJ1277	*metJ120*	argH$^+$	248	8		5	3	*glpK metJ1277 metJ120 argH*
metJ120	*metJ1277*	argH$^+$	248	1		0	1	" " " "
metJ1279	*metJ1252*	argH$^+$	248	4		3	1	*glpK metJ1279 metJ1252 argH*
metJ1252	*metJ1279*	argH$^+$	248	4		1	3	" " " "
metJ1279	*metJ93*$_{am}$	argH$^+$	248	4		3	1	*glpK metJ1279 metJ93*$_{am}$ *argH*

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of trans-ductants tested	Number of metJ$^+$ recombi-nants found	Distribution of out-side markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ1279	*metJ36*	argH$^+$	248	2		2	0	*glpK metJ1279 metJ36 argH*
metJ1279	*metJ120*	argH$^+$	248	4		3	1	*glpK metJ1279 metJ120 argH*
metJ1252	*metJ93*$_{\text{am}}$	argH$^+$	248	2		2	0	*glpK metJ1252 metJ93*$_{\text{am}}$ *argH*
metJ1252	*metJ1209*$_{\text{am}}$	argH$^+$	248	2		2	0	*glpK metJ1252 metJ1209*$_{\text{am}}$ *argH*
metJ1252	*metJ36*	argH$^+$	248	3		3	0	*glpK metJ1252 metJ36 argH*
metJ36	*metJ1252*	argH$^+$	248	2		0	2	" " " "
metJ1252	*metJ120*	argH$^+$	248	1		1	0	*glpK metJ1252 metJ120 argH*
metJ120	*metJ1252*	argH$^+$	248	2		0	2	" " " "
metJ93$_{\text{am}}$	*metJ1209*$_{\text{am}}$	argH$^+$	248	4		4	0	*glpK metJ93*$_{\text{am}}$ *metJ1209*$_{\text{am}}$ *argH*
metJ1209$_{\text{am}}$	*metJ93*$_{\text{am}}$	argH$^+$	248	6		2	4	" " " "
metJ93$_{\text{am}}$	*metJ89*$_{\text{am}}$	argH$^+$	248	3		2	1	*glpK metJ93*$_{\text{am}}$ *metJ89*$_{\text{am}}$ *argH*
metJ89$_{\text{am}}$	*metJ93*$_{\text{am}}$	argH$^+$	248	2		0	2	" " " "
metJ1209$_{\text{am}}$	*metJ89*$_{\text{am}}$	argH$^+$	248	5		4	1	*glpK metJ1209*$_{\text{am}}$ *metJ89*$_{\text{am}}$ *argH*
metJ89$_{\text{am}}$	*metJ1209*$_{\text{am}}$	argH$^+$	248	3		1	2	" " " "
metJ36	*metJ1209*$_{\text{am}}$	argH$^+$	248	5		0	5	*glpK metJ1209*$_{\text{am}}$ *metJ36 argH*

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of transductants tested	Number of metJ$^+$ recombinants found	Distribution of outside markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ120	*metJ1209*$_{\mathrm{am}}$	argH$^+$	248	4		1	3	*glpK metJ1209*$_{\mathrm{am}}$ *metJ120 argH*
metJ36	*metJ89*$_{\mathrm{am}}$	argH$^+$	200	2		0	2	*glpK metJ89*$_{\mathrm{am}}$ *metJ36 argH*
metJ120	*metJ89*$_{\mathrm{am}}$	argH$^+$	200	4		1	3	*glpK metJ89*$_{\mathrm{am}}$ *metJ120 argH*
metJ1245	*metJ89*$_{\mathrm{am}}$	argH$^+$	200	2		0	2	*glpK metJ89*$_{\mathrm{am}}$ *metJ1245 argH*
metJ36	*metJ120*	argH$^+$	372	13		10	3	*glpK metJ36 metJ120 argH*
metJ36	*metJ120*	glpK$^+$	372	10	2		8	" " " "
metJ120	*metJ36*	argH$^+$	352	6		1	5	" " " "
metJ120	*metJ36*	glpK$^+$	170	1	1		0	" " " "
metJ36	*metJ1271*	argH$^+$	248	4		4	0	*glpK metJ36 metJ1271 argH*
metJ36	*metJ1271*	glpK$^+$	248	7	1		6	" " " "
metJ1271	*metJ36*	argH$^+$	248	11		2	9	" " " "
metJ36	*metJ1245*	argH$^+$	248	3		3	0	*glpK metJ36 metJ1245 argH*
metJ1245	*metJ36*	argH$^+$	248	9		2	7	" " " "
metJ36	*metJ101*	argH$^+$	248	6		5	1	*glpK metJ36 metJ101 argH*
metJ36	*metJ101*	glpK$^+$	200	9	0		9	" " " "

APPENDIX I - continued

Donor	Recipient	Selected marker	Number of trans-ductants tested	Number of metJ$^+$ recombi-nants found	Distribution of out-side markers in metJ$^+$ recombinants			Order inferred
					glpK$^+$ argH$^-$	glpK$^-$ argH$^+$	glpK$^+$ argH$^+$	
metJ101	*metJ36*	argH$^+$	310	3		0	3	*glpK metJ36 metJ101 argH*
metJ101	*metJ36*	glpK$^+$	178	1	1		0	" " " "
metJ120	*metJ1271*	argH$^+$	200	3		3	0	*glpK metJ120 metJ1271 argH*
metJ1271	*metJ120*	argH$^+$	248	10		3	7	" " " "
metJ1271	*metJ120*	glpK$^+$	248	6	4		2	" " " "
metJ120	*metJ1245*	argH$^+$	248	9		8	1	*glpK metJ120 metJ1245 argH*
metJ1245	*metJ120*	argH$^+$	248	14		5	9	" " " "
metJ120	*metJ101*	argH$^+$	248	7		7	0	*glpK metJ120 metJ101 argH*
metJ120	*metJ101*	glpK$^+$	210	3	0		3	" " " "
metJ101	*metJ120*	argH$^+$	280	1		0	1	" " " "
metJ101	*metJ120*	glpK$^+$	240	1	1		0	" " " "
metJ1271	*metJ1245*	argH$^+$	300	4		3	1	*glpK metJ1271 metJ1245 argH*
metJ1245	*metJ1271*	argH$^+$	200	1		0	1	" " " "
metJ1245	*metJ101*	argH$^+$	248	9		7	2	*glpK metJ1245 metJ101 argH*
metJ101	*metJ1245*	argH$^+$	248	11		3	8	" " " "

APPENDIX II - Enzyme activities of ethionine sensitive *metJ/metJ* merodiploids which seem to exhibit restrictive complementation

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]		
		Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
metJ^{+}	*metJ*$^{+}$[b]	1.0	1.0	1.0
metJ1245	*metJ120*	3.7	3.9	2.1
metJ120	*metJ1245*	4.4	2.9	1.2
metJ1245	*metJ36*	2.5	3.0	1.9
metJ1245	*metJ1264*	2.6	4.3	1.5
metJ1264	*metJ1245*	1.7	8.9	1.4
metJ1245	*metJ1279*	3.7	5.3	1.3
metJ1279	*metJ1245*	6.4	8.9	1.8
metJ101	*metJ1245*	6.2	8.5	2.4
metJ1245	*metJ101*	3.4	4.6	2.5
metJ1249	*metJ101*	4.6	4.9	2.3
metJ36	*metJ101*	4.4	8.0	2.0
metJ101	*metJ1284*	6.3	7.4	1.9
metJ1284	*metJ101*	3.0	2.9	1.7
metJ148	*metJ1264*	2.2	5.9	1.6
metJ1264	*metJ148*	6.3	8.4	2.7
metJ148	*metJ1284*	2.6	4.5	1.1
metJ1284	*metJ148*	4.3	9.3	2.1
metJ1245	*metJ1256*	6.3	7.5	1.4
metJ1256	*metJ1245*	6.4	8.1	1.8

APPENDIX II - continued

Episomal *metJ* allele	Chromosomal *metJ* allele	Relative activities[a]		
		Cystathionine-γ-synthetase (*metB*)	β-cystathionase (*metC*)	ATP:Methionine *S*-adenosyltransferase (*metK*)
metJ$^+$	*metJ*$^+$[c]	1.0	1.0	1.0
metJ148	*metJ1209*$_{am}$	4.4	5.8	2.1
metJ1245	*metJ1209*$_{am}$	3.3	3.3	1.8
metJ1249	*metJ1209*$_{am}$	4.2	4.6	2.1
metJ1271	*metJ1209*$_{am}$	4.5	7.9	2.2
metJ1275	*metJ1209*$_{am}$	4.0	4.1	1.9
metJ1277	*metJ1209*$_{am}$	3.8	9.4	1.8
metJ1279	*metJ1209*$_{am}$	2.1	2.5	1.9

[a] The activity of each enzyme is expressed relative to the specific activity of the wild type (*metJ*$^+$/*metJ*$^+$) which is taken as 1.0.

[b] The chromosome was *su*$^+$. The specific activities of the *metJ*$^+$/*metJ*$^+$ *su*$^+$ strain were: cystathionine-γ-synthetase, 0.283; β-cystathionase, 0.102; and ATP:Methionine *S*-adenosyltransferase, 11.39.

[c] The chromosome was *su*^{o}. The specific activities of the *metJ*$^+$/*metJ*$^+$ *su*^{o} strain were: cystathionine-γ-synthetase, 0.175; β-cystathionase, 0.112; and ATP:Methionine *S*-adenosyltransferase, 10.76. The units of specific activities are described under "Materials and Methods."